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Kinetics of nitrification in selected Iowa soils treated with Stay-N 2000

Dwi Rovita
Iowa State University

Kinetics of nitrification in selected Iowa soils treated with Stay-N 2000

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Dwi Rovita

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Program of Study Committee:
Randy Jay Killorn, Major Professor
Andrew Manu
Tom E . Loynachan
C. Lee Burras
Russell Mullen

Iowa State University

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Graduate College
Iowa State University

This is to certify that the doctoral dissertation of

Dwi Rovita

has met the dissertation requirements of Iowa State University

Signature was redacted for privacy.

Major Professor

Signature was redacted for privacy.

For the Major Program

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1. GENERAL INTRODUCTION

DISSERTATION ORGANIZATION

This dissertation is organized into five chapters. The first chapter contains a comprehensive literature review including an overview of nitrogen in agriculture, nitrification process in soils, nitrification inhibitors, and kinetics of nitrification in soils. Chapters 2 through 4 are manuscripts describing the kinetics of nitrification in Iowa soils treated with Stay-N and will be submitted to the Communication in Soil Science and Plant Analysis Journal and Journal of Environmental Quality for publication. Each individual manuscript has an abstract, introduction, materials and methods, results and discussion, summary and conclusions. References cited within each section are presented at the end of each chapter. The last chapter is the general conclusion of the study.

LITERATURE REVIEW

Nitrogen in Agriculture

It is well accepted that nitrogen (N) is an essential element and a key component in plant growth and crop reproduction. It is an essential component of chlorophyll, proteins, and other essential molecules which form the building blocks of life. Nitrogen in soils occurs in several forms including organic compounds, ammonium (NH_4^+), nitrite (NO_2^-), nitrate (NO_3^-), nitrous oxide (N_2O), and N gas (N_2). The largest concentration of the N in surface soils is in the organic form. In addition, a large part of N in the lithosphere is in a fixed form within the earth's crust in rocks and sediments and is generally unavailable for plant uptake. The main forms of N taken up by plants are NH_4^+ and nitrate NO_3^-.

Most agricultural soils are deficient in N for the growth of crops. With the advanced development in the capacity to manufacture plant available N as commercial fertilizer in the past 50 years, there is a strong incentive to use this fertilizer to supply adequate N for crop growth. Although application of N in forms available for plant uptake is the easiest solution to dealing with N deficiency, there are certain considerations of N management that must be dealt with. They are related mainly to the escape of the N from the rooting zone after application of fertilizer to the soil, creating yield limiting N deficiencies that affect the expected yield of the crop (Aldrich, 1980; Hallberg, 1989). Also, in agro-ecosystems, where large quantities of N fertilizers are used continuously, the efficiency of their use may be low and may result in environmental pollution (Kubota et al., 1976; Feigenbaum et al., 1987; Puttanna et al., 1999; and Puttanna et al., 2001).

The most extensively used sources of N fertilizers for field crops are NH_4^+ and NH_4^+-producing compounds such as urea. Ammonium-N applied to the soil is usually oxidized rapidly to NO_3^--N by nitrifying bacteria which obtain their energy for their metabolic activities from nitrification (Bronson et al., 1989; McCarty and Bremner, 1989). Nitrate is a mobile compound and losses of NO_3^--N from the soil through leaching process lead to problems of contamination which can ultimately result in eutrophication of rivers and lakes. As a consequence, NO_3^--N can accumulate in toxic concentrations in water supplies when it enters aquifers (Embleton et al., 1981).

Traditionally, soil nitrification has been studied by incubating a quantity of soil amended with NH_4^+-N and by determining the changes in concentration of NH_4^+-N and NO_3^--N regularly (Prosser and Cox, 1982).

Nitrification in Soils

There are two major forms of N available for crop growth, NH_4^+-N and NO_3^--N and each has its own unique properties. Nitrogen is extremely dynamic in soils and the application of NH_4^+-N fertilizer leads to different ecological problems. In most agricultural soils, NO_3^--N is the primary form available to crops because applied NH_4^+-N is rapidly transformed biologically to NO_3^--N through a process called nitrification. This conversion is a two-step process primarily performed by two autotrophic bacteria (van Neil et al., 1993; U.S. EPA, 1993). The first step is the conversion of ammonium to nitrite by *Nitrosomonas spp.,*

$$NH_3 + O_2 + 2H^+ + 2e^- \rightarrow NH_2OH + H_2O \rightarrow NO_2^- + 5H^+ + 4e^-$$

and the second step is the conversion of nitrite to nitrate by *Nitrobacter spp.*,

$$NO_2^- + H_2O \rightarrow NO_3^- + 2H^+ + 2e^-$$

Both microorganisms which carrying out these reactions are able to meet their carbon needs for growth by using only carbon dioxide from the air and do not require organic or other chemically combined carbon (Myrold, 1999).

The significance of the nitrification process in soils is simple; it leads to N loss. This process is crucial to the efficiency of N fertilizers and their impact on the environment, because the net effect is a conversion of fertilizer N from a form that is not minimally subject to loss from soil (NH_4^+-N) into a form that is readily lost by leaching or denitrification (NO_3^--N). Ammonium-N is positively charged and held by the negatively charged soil particles, thus prevented from leaching. Nitrate-N, in contrast, is negatively charged and not held by soil particles. Since NO_3^--N is mobile and soluble, leaching or movement downward from the crop's rooting zone leads to environmental problems. Alternatively, NO_3^--N enters aquifers and can build up to toxic concentrations in water supplies despite denitrification that results in loss of gaseous nitrogen from the system (Hoeft, 1984).

There are many interacting factors that control the nitrification process in soils. The main factors are nitrifier populations, soil aeration, ammonium concentration, soil pH, soil and other environmental factors such as temperature and soil water potential (Myrold, 1999). Therefore, it is well accepted that nitrification proceeds under a much broader range of conditions in soils than that which might be predicted from the knowledge of the physiology of bacteria involved (Walker and Wickramasinghe, 1979).

Nitrification Inhibitors

Nitrogen management techniques that minimize fertilizer-N losses by delaying nitrification may help in situations where leaching or denitrification is a problem (Walters and Malzer, 1990; Kpomblekou-A and Killorn, 1996). For decades researchers have attempted to find specific nitrification inhibitors with the ultimate goal of commercializing these compounds for agriculture use. The initial motivation of the effort was to increase the N efficiency use by crops to maximize yield by keeping fertilizer N in its more stable NH_4^+-N form for longer period, thereby diminishing the potential for NO_3^--N losses, particularly during the early growing season. Recently, the goal has expanded to include minimizing environmental contamination by NO_3^--N leaching when it is in excess of plant needs.

Nitrification inhibitors are chemicals that reduce the rate at which NH_4^+-N is converted to NO_2^--N by interfering with the metabolism of *Nitrosomonas spp.* (Campbell and Aleem, 1965; Hauck, 1980). It is a key process in managing agricultural ecosystems with respect to the fate of N.

Frequently questions have arisen regarding the use of nitrification inhibitors with N fertilizer sources such as how nitrification inhibitors work, the potential benefits they offer, and when they most likely result in an economic return.

The literatue is replete with results on nitrification inhibitors (Gasser, 1970; Prasad et. al., 1971; Huber et al., 1977; Ladd and Jackson, 1982; McCarty and Bremner, 1989), as well as review of the performance of nitrification inhibitors in the Midwest of USA (Nelson and Huber, 1980).

A number of compounds have been shown to inhibit nitrification in laboratory and field studies. Nitrapyrin [2-*chloro*-6-(*tricholoromethyl)-pyridine*], developed by the Dow Chemical Company is the most widely tested nitrification inhibitor (Okey et al., 1996). It is commercially available under the trade name of N-Serve Nitrogen Stabilizer®, and has U.S. Environmental Protection Agency approval for use on cropland in the United States (Goring, 1962; Goos, 1985). Many investigators have studied the performance of nitrapyrin as a nitrification inhibitor. Its effectiveness appears to vary from region to region and is subject to modification by many factors including the capacity of the inhibitors to preserve N in NH_4^+-N (Hergert and Wiese, 1983; Nelson and Huber, 1983; Onken 1983; Papendick and Engibous, 1983; and

Touchton and Boswell, 1983). N-serve is an extremely volatile product and can be easily lost into the atmosphere if not incorporated into the soil. Due to this high volatility, nitrapyrin has recently been reformulated with the name of Stay-N 2000 by Platte Chemical Co (Greeley, CO). Unlike nitrapyrin that used xylene as its solvent and carrier with a flash point of 79°F, Stay-N uses an Exxon-Mobil solvent, Aromatic 200, with a flash point of 200°F. This compound is emulsifiable and less volatile than nitrapyrin.

Kinetics Study of Nitrification in Soils

Interest in nitrification inhibitors to delay the nitrification process and to minimize the loss of N from the rooting zone by maintaining applied N in the ammonium form, and to diminish N losses into surface and groundwater that may cause environmental contamination has been high.

The nitrification process is a major control point in the N cycle and rates have been intensively studied in a variety of ecosystems. Many authors have discussed the NH_4^+, NO_2^- and NO_3^- reaction kinetics in some detail (Leggett and Iskandar, 1980; Brennen and Argaman, 1990; Hanaki, et al., 1990; U.S. EPA, 1993). Although nitrification has been the subject of many investigations, not until recently have efforts been made to study this important process in soils. However, there are still very few publications that describe the reaction kinetics of nitrification in soils. Because kinetic constants from pure cultures are unlikely to represent the patterns for the soil community, several attempts have been made to simulate this process in soils (Sabey, 1959; Meikle, 1979; Bhat et al., 1980; Tanji, 1982; Gilmour, 1984; Du Preez and Laubscher, 1991). Considerable effort has been devoted to understanding the biochemical oxidation of ammonium by autotrophic microorganisms and the modes of action for specific inhibitors. In addition, mechanistic models of nitrification to estimate the amount of nitrate occurring in soils require the understanding of kinetic parameters from a range of ecosystems. These concepts should also help evaluate the influence of several factors that are thought to control or affect the inhibition of nitrification in soils related to soil and inhibitor properties (Keeney, 1980).

As many other biological reactions, the course of nitrification in soils is characterized by a sigmoidal curve with a delay period during which the number of bacteria increase, a maximum rate phase and a termination rate phase due the NH_4^+-N depletion in soil (Sabey et al., 1959; Hadas et al.,1986). In different instances, nitrification rates have been modeled as zero order equations, in which the rate of nitrification is independent of substrate concentration, with a rate of *K* and a correction for the time for a delay period (Sabey et al., 1969; Beek and Frissel, 1973; Addiscott, 1983). First order equations, in which the rate of nitrification decreases with depletion of NH_4^+-N also have been used to describe the nitrification process in water and in nitrate transport models (Mehran and Tanji, 1974; Cameron and Kowalenko, 1976). In more detailed models, first order functions based on Michaelis-Menten kinetics that describe the rate of nitrification as a function of substrate concentration and biological parameters were also used (Ardakani et al., 1974; van Veen and Frissel, 1981; Malhi and McGill, 1982). Although these equations describe all the phases of nitrification, the necessary information related to their microbiological parameters makes them difficult to use for the prediction of the nitrification process in soils (Hadas, et al., 1986). Therefore, additional information is necessary to relate the nitrification inhibition by Stay-N with time in a wide range of soils amended with N.

This study was initiated (1) to evaluate the performance and effectiveness of Stay-N 2000 to delay the nitrification of soil-N and applied NH_4^+-N in Iowa soils and (2) to study the kinetic parameters by comparing changes in maximum nitrification rate (K_{max}), duration of lag period (t'), period of maximum nitrification (Δt), and duration of lag plus maximum nitrification period (t_s) as affected by different soil properties, incubation temperatures, soil water potential, fertilizer rates, inhibitor rates and in the presence of heavy metals.

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CHAPTER 2. KINETICS OF NITRIFICATION IN SELECTED IOWA SOILS TREATED WITH STAY-N

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D. Rovita and R. Killorn

ABSTRACT

Nitrification rates were investigated in two Iowa soils that differed in organic carbon contents, pH, and texture. This study was conducted to evaluate nitrification inhibition by reformulated nitrapyrin [2-chloro-6-(tricholoromethyl) pyridine] or Stay-N 2000 developed by Platte Chemical Co (Greeley, CO) applied to 10 g of surface soils representing Clarion and Okoboji series treated with a solution containing 2 mg of N as ammonium sulfate $(NH_4)_2SO_4$. A nonlinear regression was used to determine nitrate accumulation. First order equations were used to calculate the maximum nitrification rate (K_{max}), the duration of lag period (t'), the period of maximum nitrification (Δt), and the termination period of maximum nitrification (t_s). From our incubation study, the course of nitrification varied among soils used. Stay-N 2000 appeared to be the better inhibitor to extend the period of maximum nitrification compared to nitrapyrin; and as good as nitrapyrin in reducing maximum nitrification rates in both soils studied. The effectiveness of a given rate of Stay-N 2000 may be enhanced in soils with high pH and organic C, such as in the Okoboji soil. At the rate of 12 μg a.i. g^{-1} soil or three times higher than recommended rate, Stay-N 2000 reduced the maximum nitrification rate an appreciable amount in the Okoboji soil. Nitrification rates in both soils were also affected by the rates of N

applied to these soils. The NH_4^+-N depletion was fastest when 0.5 mg N was applied to the soils compared to 2 mg N. The delaying effect of nitrification seemed to be associated with high concentration of NH_4^+-N. As one important factor in regulating nitrification is substrate availability, particularly NH_4^+-N, which suggests that the availability of this substrate is often limiting the growth of nitrifiers. Nitrification proceeded rapidly in the Okoboji soil compared to the Clarion soil. The overall process showed that the more the NO_3^--N accumulation, the higher the maximum rate of nitrification (K_{max}) and the longer the delay period (t').

INTRODUCTION

Rapid oxidation of ammonium (NH_4^+) into nitrate (NO_3^-) due to nitrification in agricultural soils has a major environmental impact in many countries (Kurtz, 1983; Jarvis et al., 1995; Di and Cameron 2002). The NO_3^- produced from this process is susceptible to loss by leaching and denitrification and may contribute to NO_3^- pollution of ground and surface waters. To achieve N fertilizer efficiency for crop production and to minimize environmental pollution from NO_3^- , widespread research carried out to find compounds that can be used as amendments to delay the nitrification process that will lengthen the time N stays in the in the NH_4^+-N form.

A large number of chemicals have been used to inhibit nitrification (Bundy and Bremner, 1973; McCarty and Bremner, 1989). Nitrapyrin [2-*chloro*-6-(*tricholoromethyl)-pyridine*], manufactured by the Dow Chemical Company under the trade name "N-Serve" is the most widely evaluated nitrification inhibitor (Hauck, 1980; Walters and Malzer, 1990; Bronson *et al.*, 1992; Okey et al., 1996). It has U.S. Environmental Protection Agency approval for use on cropland in the United

States (Goring, 1962a; Goos, 1985). N-serve is a volatile product and can be easily lost if not incorporated into the soil. In addition, N-serve needs an emulsifying agent in order to ensure complete dissolution. Recently, nitrapyrin has been reformulated with the name of Stay-N 2000 by Platte Chemical Co (Greeley, CO). Unlike nitrapyrin that used xylene as its solvent and carrier with a flash point of 79°F, Stay-N uses an Exxon-Mobil solvent, Aromatic 200, with a flash point of 200°F. This compound is emulsifiable in water and is less volatile compared to nitrapyrin.

Understanding the kinetics of nitrification is allowing investigation of the interacting physical, chemical and biological factors involved in controlling the mechanism of this process in the soil. The kinetics of nitrification have been studied in pure and mixed enrichment culture, and modeled in the soil environment (Prosser, 1989). As many other biological reactions, the course of nitrification in soils is characterized by a sigmoidal curve with a delay period during which the number of bacteria increases, a maximum rate phase and a termination phase due to depletion of the NH_4^+-N (Sabey et al., 1959; Hadas et al., 1986). Nitrification rates have been modeled as zero order kinetics, in which the rate of nitrification is independent of substrate concentration, (Sabey et al., 1969; Beek and Frissel, 1973; Addiscott, 1983) and first order equations, in which the rate of nitrification decreases with depletion of NH_4^+-N (Mehran and Tanji, 1974; Cameron and Kowalenko, 1976). In more detailed models, a microbiological approach based on Michaelis-Menten kinetics has also been used to describe the rate of nitrification as a function of substrate concentration and biological parameters (Ardakani et al., 1974; van Veen and Frissel, 1981; Malhi and McGill, 1982). Although these equations describe all the phases of nitrification, the necessary information related to their microbiological parameters makes it difficult to use them for the prediction of the nitrification process in soils (Hadas et al., 1986). Therefore, additional information is necessary to relate the nitrification inhibition by Stay-N 2000 with time in a wide range of soils amended with N.

The objective of this study was (1) to evaluate the performance and effectiveness of Stay-N 2000 in delaying the nitrification of applied NH_4^+-N in Iowa soils, and (2) to characterize the nitrification process quantitatively by using a non linear regression equation in order to be able to calculate the approximate kinetic parameters of maximum nitrification rate (K_{max}), duration of lag period (t'), period of maximum nitrification (Δt), and duration of lag plus maximum nitrification period (t_s).

MATERIALS AND METHODS

Soils

The soils used (Table 1) were surface (0-15 cm) samples of representative Clarion and Okoboji soil series collected in the summer of 2002 from cultivated soils near Ames, Iowa. These soils were selected to obtain ranges in pH (6.2 - 8.1), organic carbon (OC) (23.6 - 41.7 g kg^{-1}), total N (1.9 - 3.5 g kg^{-1}), and texture (235 - 327 g kg^{-1} clay and 266 - 404 g kg^{-1} sand). Each soil was air-dried and crushed to pass a 2 mm screen. In the analysis reported in Table 1, pH was determined with a glass electrode (soil:water or 0.01 M $CaCl_2$ ratio 1:2.5). Total C and N were determined using a CHN-2000 Leco. Organic C was obtained by previously determining the $CaCO_3$ content using a Fluke 70 III multimeter in order to be able to calculate the inorganic C (%$CaCO_3$ x 0.1199), then the number was subtracted from the total C. Particle size distribution was determined by the pipette method of Kilmer and Alexander (1949).

Procedures

There are three experiments involved in this study:

Effect of Inhibitors on Nitrification of Ammonium Sulfate Added to Iowa Soils

A soil sample (10 g) was placed in a 118-mL French square bottle and treated with 2 mL of solution containing 2 mg N and 4 μg a.i. g^{-1} soil of Stay-N 2000 or nitrapyrin. Stay-N 2000 was obtained from Platte Chemical Co., Greeley, Colorado while nitrapyrin was obtained from DowElanco Chemical Co.

Effect of Stay-N 2000 Rates on Nitrification in Iowa Soils

A soil sample (10 g) was placed in a 118-mL French square bottle and treated with 2 mL of solution containing 2 mg N and 4, 8, or 12 μg a.i. g^{-1} soil of Stay-N 2000.

Kinetics of Nitrification at Different Rates of N Added to Iowa Soils

A soil sample (10 g) was placed in a 118-mL French square bottle and treated with 2 mL of solution containing 0.5, 1, or 2 mg N and 4 μg a.i. g^{-1} soil of Stay-N 2000.

To each experiment, additional deionized water was added to bring the soil moisture content to 60% of the water holding capacity. All bottles were then covered with parafilm perforated in the center for aeration and placed in an incubator at 20 ± 1°C and 90 % r.h., for 0, 7, 15, 30, 45, and 60 d. The amount of water lost during incubation was replaced if the loss exceeded 1 g by measuring the water content of the sample gravimetrically weekly.

At the end of the incubation period, each sample was extracted with 50 mL of 2 M KCl solution. The suspension was filtered through a Whatman no. 1 filter paper after equilibration for 1 h. The extract was analyzed for (NO_3^- + NO_2^-)-N and NH_4^+-N using a QuickChem AE Automated Ion Analyzer (Lachat Instruments, 1990).

The nitrification rate equations were calculated using the nonlinear procedure of SAS (SAS Institute, 2003).

Kinetic Parameters

The nonlinear regression by Hadas et al. (1986) was used to estimate the accumulation of NO_3^--N, which upon integration gives a sigmoidal curve:

$$NO_3^- = a/\{1 + (a/[NO_3^-]_0 - 1)\exp(-ak[t - t_0])\} \quad [1]$$

where NO_3^- is NO_3^- accumulation (mg NO_3^--N kg^{-1}) at a particular time, a is the asymptotic value of NO_3^- and $(NO_3^-)_0$ is the initial value of NO_3^- at time zero (t_0), k is a constant, and t_0 is the initial time, which equals zero. The maximum nitrification rate (K_{max}) was estimated by using the equation developed by Sabey et al. (1959) and Hadas et al. (1986) as the maximum slope of eq [1], at the inflection point (when NO_3^- = a/2):

$$K_{max} = k(a^2/4) \quad [2]$$

The duration of delay period (t') was calculated from the formula described by Sabey et al. (1959) and Hadas et al. (1986):

$$t' = (1/ak)\ln[a/(NO_3^-)_0 - 1] + [(NO_3^-)_0 - a/2]/K_{max} \quad [3]$$

The duration of period of maximum nitrification (Δt) was calculated by using the Laubscher et al. (1990) equation.

$$\Delta t = 2[a/2 - (NO_3^-)_0]/K_{max} \quad [4]$$

The termination period of maximum nitrification rate (t_s), can be determined after obtaining the duration of delay period (t') and the duration of period of maximum nitrification (Δt) as it is described by Laubscher et al. (1990) equation.

$$t_s = t' + \Delta t \quad [5]$$

RESULTS AND DISCUSSIONS

Data on NH_4^+-N recovered during the incubation period are presented in Tables 2, 3, and 4. Figures 1, 3, and 5 show the accumulation of NO_3^--N during the incubation period. All figures obtained in our experiments are sigmoidal when cumulative NO_3^--N production was plotted against incubation time. We used Hadas et al. (1986) equation to describe the course of nitrification in the two soils studied. The kinetic parameters calculated from this equation are presented in Figures 2, 4, and 6. Nitrification activities of the experimental soils varied with the inhibitors used, the rates of Stay-N 2000 applied, and the rates of N applied to the soils. The results therefore will be discussed separately.

Effect of Inhibitors on Nitrification of Ammonium Sulfate Added to Iowa Soils

We conducted this experiment using Stay-N 2000 and nitrapyrin. Table 2 shows that the disappearance of NH_4^+-N added to both soils was very slow in the Clarion soil compared to the Okoboji soil. We assumed that a considerable portion of it was fixed by clay. Cameron and Kowalenko (1976) reported that ≈ 50% NH_4^+ added to the soils was fixed within 6 to 10 d. However, no direct measurements of fixed NH_4^+ were taken in our experiment to prove this assumption. In addition, besides soil texture (235 g kg^{-1} clay and 404 g kg^{-1} sand), lower pH and OC content in the Clarion soil compared to the Okoboji soil may also contribute to slow NH_4^+ disappearance. Although reviews relevant to fertilized soils (Pesek et al., 1971; Russell, 1973; Schmidt, 1982) reported that nitrification rates in soils are little affected by soil pH within the range of 5.5 and 8, it is well established that nitrification is relatively slow at pH values between 4.7-6.0 (Alexander, 1965; Hendrickson and Keeney, 1979; Touchton et al., 1979; Sahrawat, 1982; Schmidt, 1982). This was because the rate of recovery of surviving nitrifiers is higher at high pH values (Goring, 1962b) and parts of this effect may be due to the presence of carbonates at higher pH values which provide a source of CO_2 needed for the autotrophic organisms involved (Kinsbursky and Saltzman, 1990). These effects, however, have not been linked to problems related to nitrification rates in the soil studied. Organic carbon, on the other hand, is another factor affecting nitrification in soils. Rapid depletion of NH_4^+-N and high accumulation of NO_3^--N were found in the Okoboji soil (Table 2). Keeney (1980) concluded in his study that accumulation of nitrate increased as soil organic C increased.

Both nitrapyrin and Stay-N 2000 reduced soil nitrification as is indicated by the reduced accumulation of NO_3^--N in soils (Fig. 1). The kinetic parameters of soil nitrification treated with Stay-N 2000 and nitrapyrin are presented in Fig 2. The rate of NO_3^--N formation varied not only with soils but also with the type of inhibitors. The nitrification proceeded most rapidly in the Okoboji soil with the maximum rate of nitrification (K_{max}) of 28 mg kg^{-1} d^{-1} in the control soils, 6 mg kg^{-1} d^{-1} in the treated soil with nitrapyrin and 5 mg kg^{-1} d^{-1} in the treated soil with Stay-N 2000. The rate was slower in the Clarion soil which is slightly acidic and lower in organic C than the Okoboji soil with (K_{max}) of 14 mg kg^{-1} d^{-1} in the control soil and 1 mg kg^{-1} d^{-1} in the treated soil with nitrapyrin and 0.9 the treated soil with Stay-N 2000. The lower effectiveness of both Stay-N 2000 and nitrapyrin in the Okoboji soil seemed to be related to the organic C content of this soil. Studies by Goring (1962a), Briggs (1975), and Hendrickson and Keeney (1978) indicated that organic matter is a major sorbing component of inhibitors applied to the soils. Other studies also showed decreased effectiveness of nitrapyrin with increases in organic matter content (Bundy and Bremner, 1973; Lewis and Stefanson, 1975).

The extension of t' due to the addition of Stay-N 2000 was as a little as 5 d in the Okoboji soil and as high as 10 d in the Clarion soil. On the other hand, addition of nitrapyrin extended the t' of the Okoboji soil to 4 d and to 6 d in the Clarion soil. Although addition of nitrapyrin did not significantly increase t' with respect to the control treatment in Clarion soil (Fig. 2); it did extend the period of maximum nitrification (Δt) to 30 and 25 d in Clarion and Okoboji soils, respectively. Stay-N 2000 extended the Δt to 51 and 24 d in the Clarion and Okoboji soils, respectively. Figure 2 also shows the increased t_s with the addition of inhibitors. The estimated asymptotic values of the Clarion soil were 134 and 140, respectively, when this soil was treated with Stay-N 2000 and nitrapryrin; and 199 and 225, respectively, in the Okoboji soil when this soil was treated with Stay-N 2000 and nitrapryrin. Stay-N

2000 appeared to be the better inhibitor in extending the period of maximum nitrification compared to nitrapyrin; and as good as nitrapyrin in reducing maximum nitrification rates in both soils studied.

Effect of Stay-N 2000 Rates on Nitrification in Iowa Soils

Some studies showed that increasing soil organic matter content decreases the effectiveness of nitrification inhibitors. We conducted experiments to determine the rates of Stay-N 2000 required for total inhibition nitrification in two Iowa soils that differ in soil organic C and soil pH (Table 1). Four different rates, 0, 4, 8 and 12 μg a.i. g^{-1} soil of Stay-N 2000 were applied to soils. Without Stay-N 2000 application, the disappearance of NH_4^+-N in the Okoboji soil was faster than that in the Clarion soil (Table 3). The higher the rate of Stay-N 2000 applied, the slower the disappearance of NH_4^+-N, especially in the Okoboji soil. However, in the Clarion soil, the rate of NH_4^+-N disappearance did not seem to be different between 8 and 12 μg a.i. g^{-1} soil of Stay-N 2000. Although some investigators reported that high rates of nitrification inhibitor such as nitrapyrin may increase the potential for NH_4^+-N toxicity to some crops (McKell and Whalley, 1964; Riley and Barber, 1970; Sander and Barker, 1978), our findings suggest that higher rates of Stay-N 2000 are required to reduce NO_3^--N accumulation (Fig. 3) and rapid NH_4^+-N depletion (Table 3) in the soil with high pH and soil organic matter contents.

The maximum rates of nitrification (K_{max}) were 13, 0.7, 0.4, and 0.3 mg kg^{-1} d^{-1} at the rates of 0, 4, 8 and 12 μg a.i. g^{-1} soil of Stay-N 2000, respectively in the Clarion soil and 26, 5, 2, and 0.8 mg kg^{-1} d^{-1} at the rates of 0, 4, 8 and 12 μg a.i. g^{-1} soil of Stay-N 2000, respectively in the Okoboji soil. It seemed that Stay-N

2000 reduced the maximum nitrification rate an appreciable amount in the Okoboji soil when 12 µg a.i. g^{-1} soil of Stay-N 2000 was applied to this soil. As Hendrickson and Keeney (1979) reported, sorption of nitrapyrin by soil organic matter is a major factor decreasing the effectiveness of this inhibitor, therefore the effectiveness of Stay-N 2000 may be enhanced in soils with high pH and organic C when a high concentration of Stay-N 2000 is applied to the soil.

Accumulation of NO_3^--N was affected by the addition of Stay-N 2000 (Fig. 3). It shows the extension of the lag period (t') corresponding to a population growth of nitrifiers (Morrill and Dawson, 1967), the period of maximum nitrification, and the period of termination (Laubscher et al., 1990). The lag period f of the soil without inhibitor varied between two soils (Fig. 4). The variation of (t') is probably a function of soil pH (Morill and Dawson, 1967); the higher the pH value, the shorter the (t'). When Stay-N 2000 was added, it lengthened the t' in both soils. In the Clarion soil, the increasing rates of Stay-N 2000 added to this soil extended the t' to 3, 4, and 5 d compared to the control treatment. In the Okoboji soil on the other hand, even though it extended the t' compared to control soils, the increasing rates of Stay-N 2000 did not seem to extend the t' significantly. Stay-N 2000 is capable of extending the period of maximum nitrification (Δt) as well as the period of termination (t_s) in both soils. Figure 4 shows that the higher the rates, the longer the Δt as well as t_s of both soils. As shown in Figure 4, the highest rate of Stay-N 2000 was more effective than the lower rates.

Kinetics of Nitrification at Different Rates of N Added to Iowa Soils

Different rates of N added to soils affected nitrification rates as measured by NO_3^--N production (Fig. 5) and NH_4^+-N disappearance (Table 4). Table 4 shows that the higher the N rate, the slower the disappearance of NH_4^+-N in both soils. The disappearance of NH_4^+-N is slower in control and treatments of the Clarion soil compared to the Okoboji soil. The N rate treatments affected the kinetics of nitrification in both soils (Fig. 6). The N applied to soils was 0.5, 1, and 2 mg N in the form of $(NH_4)_2SO_4$. The lowest NO_3^--N accumulation occurred in the Clarion soil when 0.5 mg N was applied. The concentration is even lower than the control soil when additional Stay-N accompanied the N. In the Okoboji soil, however, the accumulation of NO_3^--N proceeded more rapidly than in Clarion soil even though Stay-N 2000 was added to this soil. As in our previous experiment, this phenomenon was probably due to high soil organic C content, a major sorbing component of the inhibitor used (Hendrickson and Keeney, 1978).

Application of Stay-N 2000 markedly altered the course of nitrification as indicated by the nitrification parameters (Fig. 6). The maximum rate of nitrification (K_{max}) in the control and treated Clarion soil was 4 and 0.5 mg kg^{-1} d^{-1} NO_3^--N, respectively, when the soil was treated with 0.5 mg N, followed by 8 and 0.7 mg kg^{-1} d^{-1} NO_3^--N respectively when the soil was treated with 1 mg N, and 13 and 0.7 mg kg^{-1} d^{-1} NO_3^--N, respectively, when the soil was treated with 2 mg N. The K_{max} in the control and treated Okoboji soil was 5 and 2 mg kg^{-1} d^{-1} NO_3^--N when the soil was treated with 0.5 mg N, followed by 10 and 4 mg kg^{-1} d^{-1} NO_3^--N, respectively, when the soil was treated with 1 mg N, and 22 and 5 mg kg^{-1} d^{-1} NO_3^--N, respectively, when the soil was treated with 2 mg N. Different rates of N applied to the soils extended the delay period (t') of nitrification compared to the control treatment in both soils. The t' of both the Clarion and Okoboji soils extended to 2, 3, and 4 d when the samples were treated with 0.5, 1, and 2 mg of N, respectively. Although application of different rates of N gave a similar number of days of the delay period in both soils, it extended the period of maximum nitrification (Δt) as well as the termination period (t_s) of both soils at a different number of days. The Δt extended to 6, 8, and 29 d in the Clarion soil; and to 10, 11, and 23 d in the Okoboji soil when both soils were treated with 0.5, 1, and 2 mg of N, respectively. Some investigators have noted the delaying effect of nitrification associated with a high concentration of NH_4^+-N. Broadbent et al. (1957); McIntosh and Frederick (1958) concluded that nitrification occurred rapidly and completely when NH_4^+-N added to soils was low. The most important factor in regulating nitrification is substrate availability, particularly NH_4^+-N, which suggests that the availability of this substrate is often limiting the growth of nitrifiers (Myrold, 1999).

SUMMARY AND CONCLUSIONS

These studies indicate that application of Stay-N 2000 and nitrapyrin to NH_4^+-N amended Iowa soils can slow down the rate of nitrification. Both inhibitors reduced the maximum rate of nitrification, extended the delay period of nitrification, and subsequently extended the period of maximum nitrification and the period of termination of nitrification. The Stay-N 2000, however, appeared to be the better inhibitor in extending the period of maximum nitrification compared to nitrapyrin; and as good as nitrapyrin in reducing maximum nitrification rates in both soils studied.

The rate of NO_3^--N formation was affected by the rates of Stay-N 2000 applied to both soils. Stay-N 2000 at the rate of 12 μg a.i. g^{-1} soil reduced the maximum nitrification rate an appreciable amount in the Okoboji soil with higher pH and organic C compared to the Clarion soil. This rate was three times higher than the recommended rate of Stay-N 2000. Thus, the effectiveness of a given rate of Stay-N 2000 may increase on soils with high pH and organic C. Stay-N 2000 is capable of extending the period of maximum nitrification (Δt) as well as the period of termination (t_s) in both soils. The higher the rates, the longer the Δt as well as t_s of both soils; and the highest rate of Stay-N 2000 was more effective than the lower rates in reducing nitrification.

Nitrification rates in both the Clarion and Okoboji soils were affected by the rates of N applied to these soils. The nitrification proceeded rapidly in the Okoboji soil compared to the Clarion soil. The overall process, however, showed that the higher the N rates, the more the NO_3^--N accumulation, the higher the maximum rate of nitrification (K_{max}), the longer the delay period (t'), the longer the period of maximum nitrification (Δt) as well as the termination period of nitrification (t_s). As one of the most important factors in regulating nitrification is substrate availability, particularly NH_4^+-N, our findings suggest that the low availability of this substrate limits the growth of nitrifiers.

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Table 1. Initial properties of soil used

Soil series	pH		Organic	Total	Inorganic N		Clay	Sand
	H_2O	$CaCl_2$	C	N	NH_4-N	NO_3-N		
			----g kg^{-1} soil---		--mg kg^{-1} soil--		------g kg^{-1}------	
Clarion	6.2	5.8	23.6	1.9	2	4	235	404
Okoboji	8.1	7.6	41.7	3.5	7	6	327	266

Table 2. Effect of incubation time on NH_4^+-N recovered after addition of $(NH_4)_2SO_4$ to soils

Soil and inhibitor	NH_4^+-N recovered					
	Incubation time					
	0 d	7 d	15 d	30 d	45 d	60 d
	---------------------------μg g-1 soil------------------------					
Clarion						
No inhibitor	230	134	87	18	12	6
Nitrapyrin	232	210	202	198	186	170
Stay-N	232	224	218	210	204	175
Okoboji						
No inhibitor	240	166	25	0	0	0
Nitrapyrin	238	171	161	131	75	7
Stay-N	237	180	169	140	85	32

Table 3. Effects of different rates of Stay-N 2000 on NH_4^+-N recovered

Soil and Stay-N 2000	NH_4^+-N recovered					
	Incubation time					
	0 d	7 d	15 d	30 d	45 d	60 d
	----------µg g-1 soil----------					
Clarion						
No inhibitor	220	140	124	101	24	6
4 µg a.i. g^{-1} soil	224	218	214	195	184	145
8 µg a.i. g^{-1} soil	220	211	207	190	186	166
12 µg a.i. g^{-1} soil	223	210	207	192	187	165
Okoboji						
No inhibitor	230	43	0	0	0	0
4 µg a.i. g^{-1} soil	238	60	161	131	75	7
8 µg a.i. g^{-1} soil	230	144	140	131	105	71
12 µg a.i. g^{-1} soil	231	187	156	135	122	117

Table 4. Effects of different rates of N on NH_4^+-N recovered in Iowa soils treated with Stay-N 2000

Soil, inhibitor and nitrogen	NH_4^+-N recovered (mg kg^{-1}) Incubation time 0 d	7 d	15 d	30 d	45 d	60 d
	------µg g-1 soil------					
Clarion						
0.5 mg N	69	55	0	0	0	0
0.5 mg N + Inh	67	59	57	54	48	44
1 mg N	148	58	0	0	0	0
1 mg N + Inh	142	110	108	95	92	84
2 mg N	244	173	47	0	0	0
2 mg N + Inh	242	207	193	175	169	157
Okoboji						
0.5 mg N	77	0	0	0	0	0
0.5 mg N + Inh	70	18	8	0	0	0
1 mg N	133	24	0	0	0	0
1 mg N + Inh	135	43	31	12	0	0
2 mg N	226	22	0	0	0	0
2 mg N + Inh	227	160	101	89	59	13

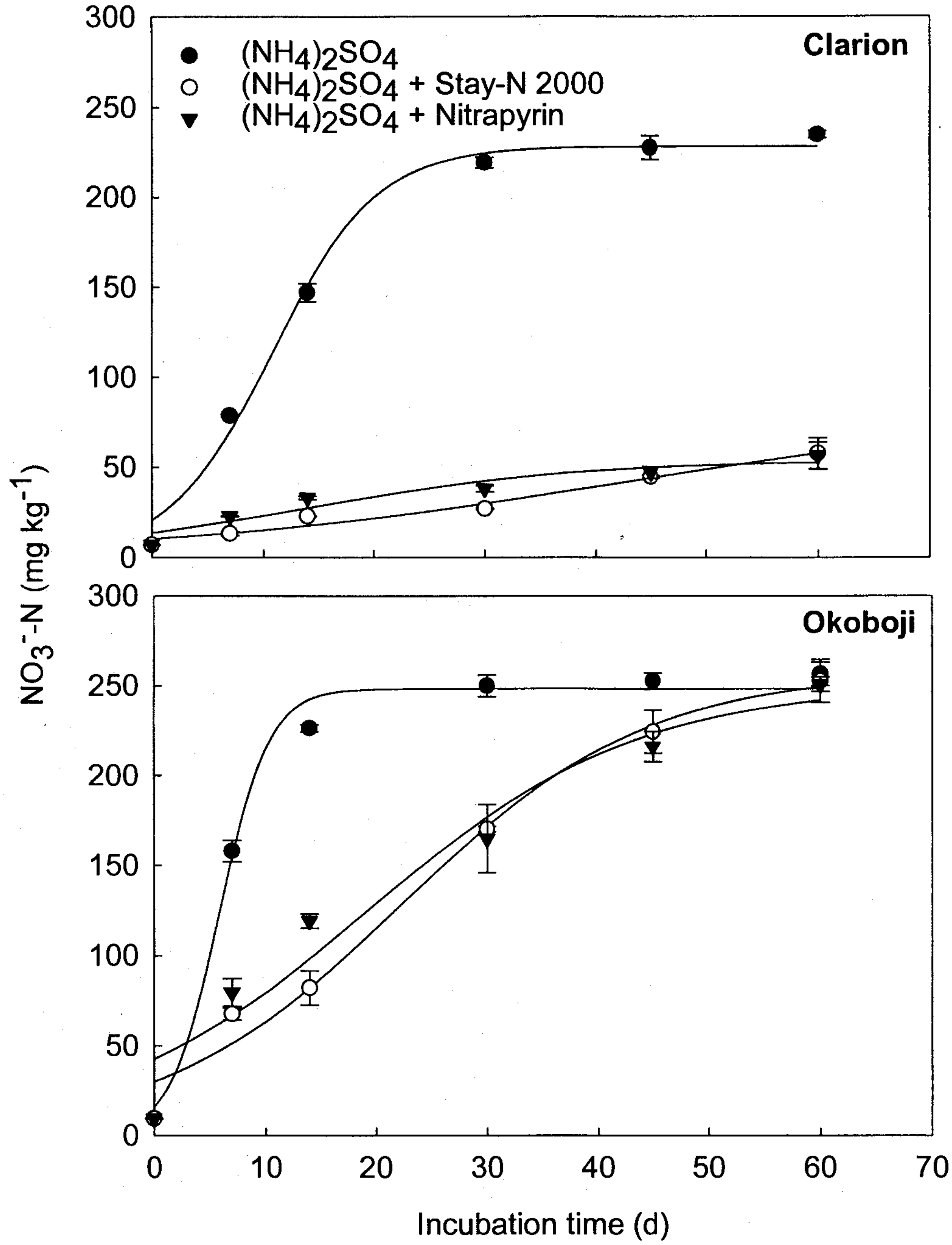

Figure 1. Nitrate-N accumulation in soils amended with $(NH_4)_2SO_4$ in the presence and absence of inhibitors.

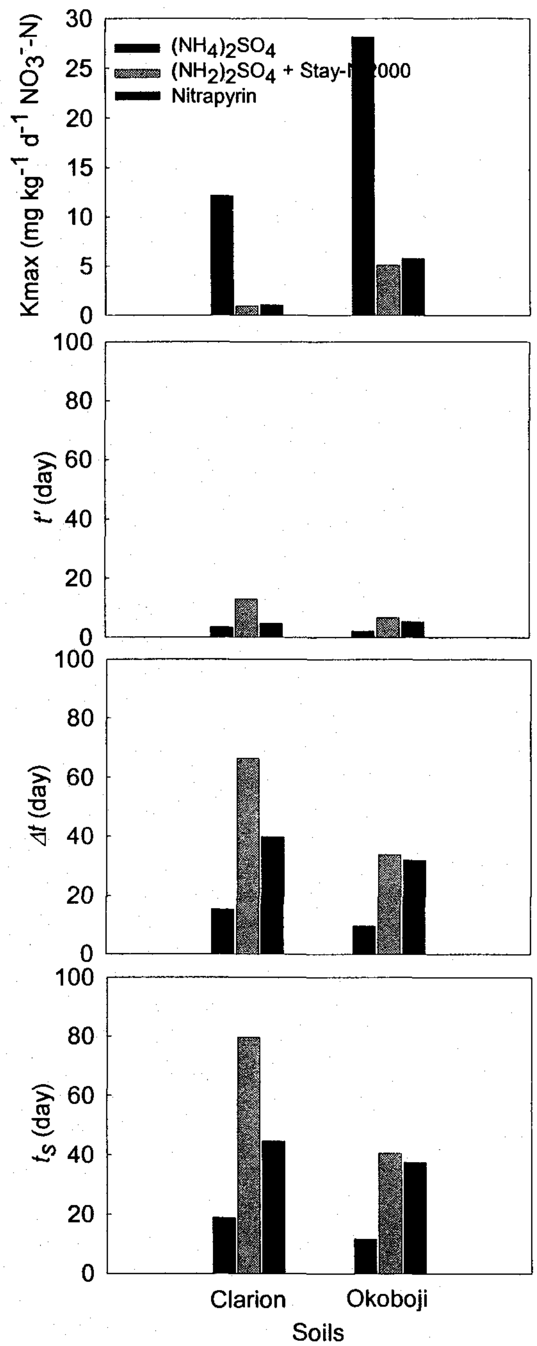

Figure 2. Calculated nitrification parameters of added $(NH_4)_2SO_4$ in the presence and absence of inhibitors.

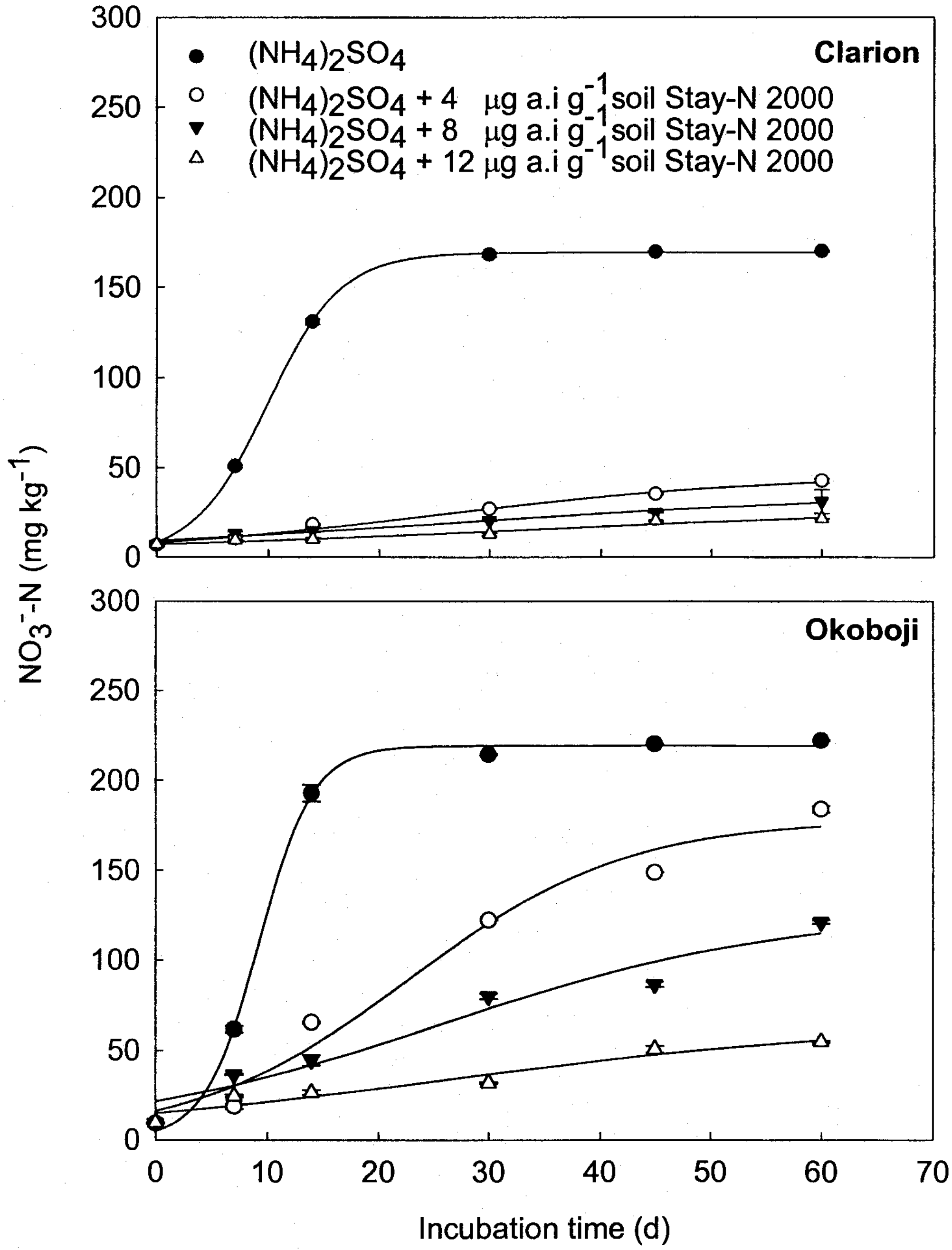

Figure 3. Effects of Stay-N 2000 rates on the accumulations of NO_3^--N in two Iowa soils.

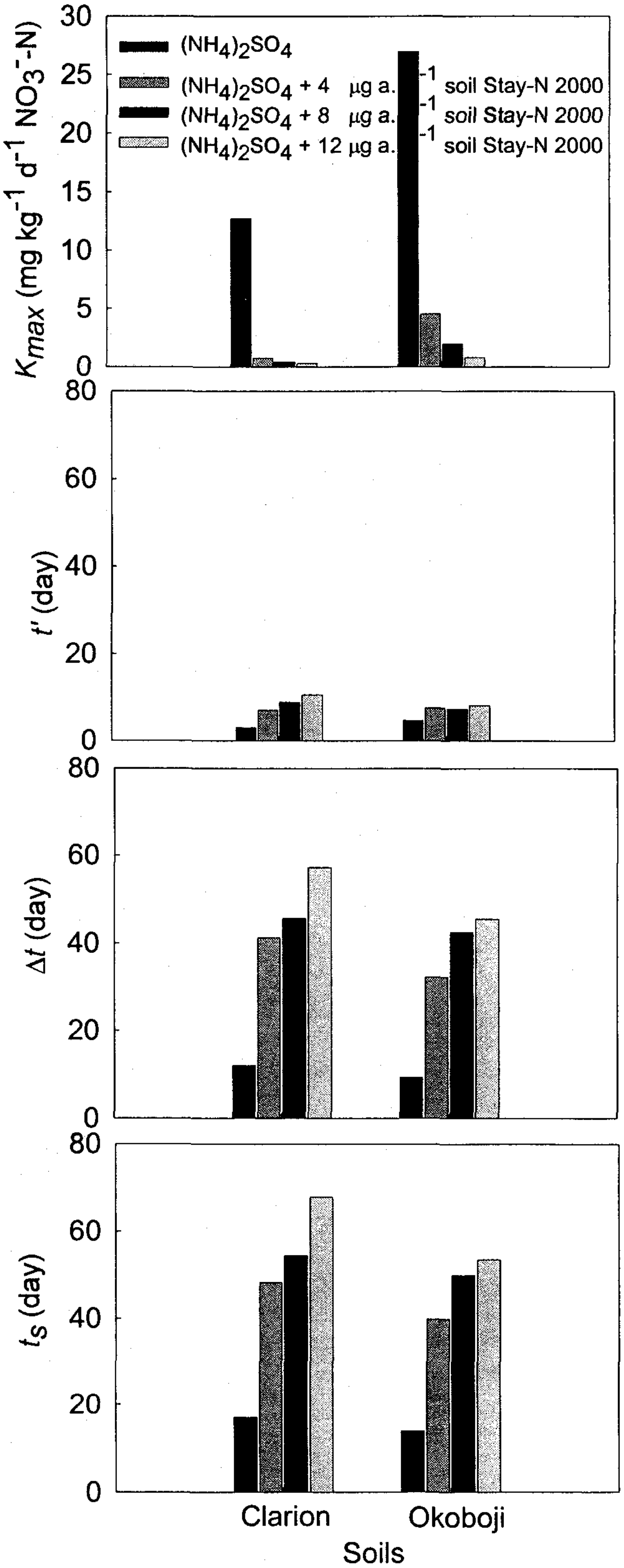

Figure 4. Calculated nitrification parameters of added $(NH_4)_2SO_4$ as affected by different rates of Stay-N 2000.

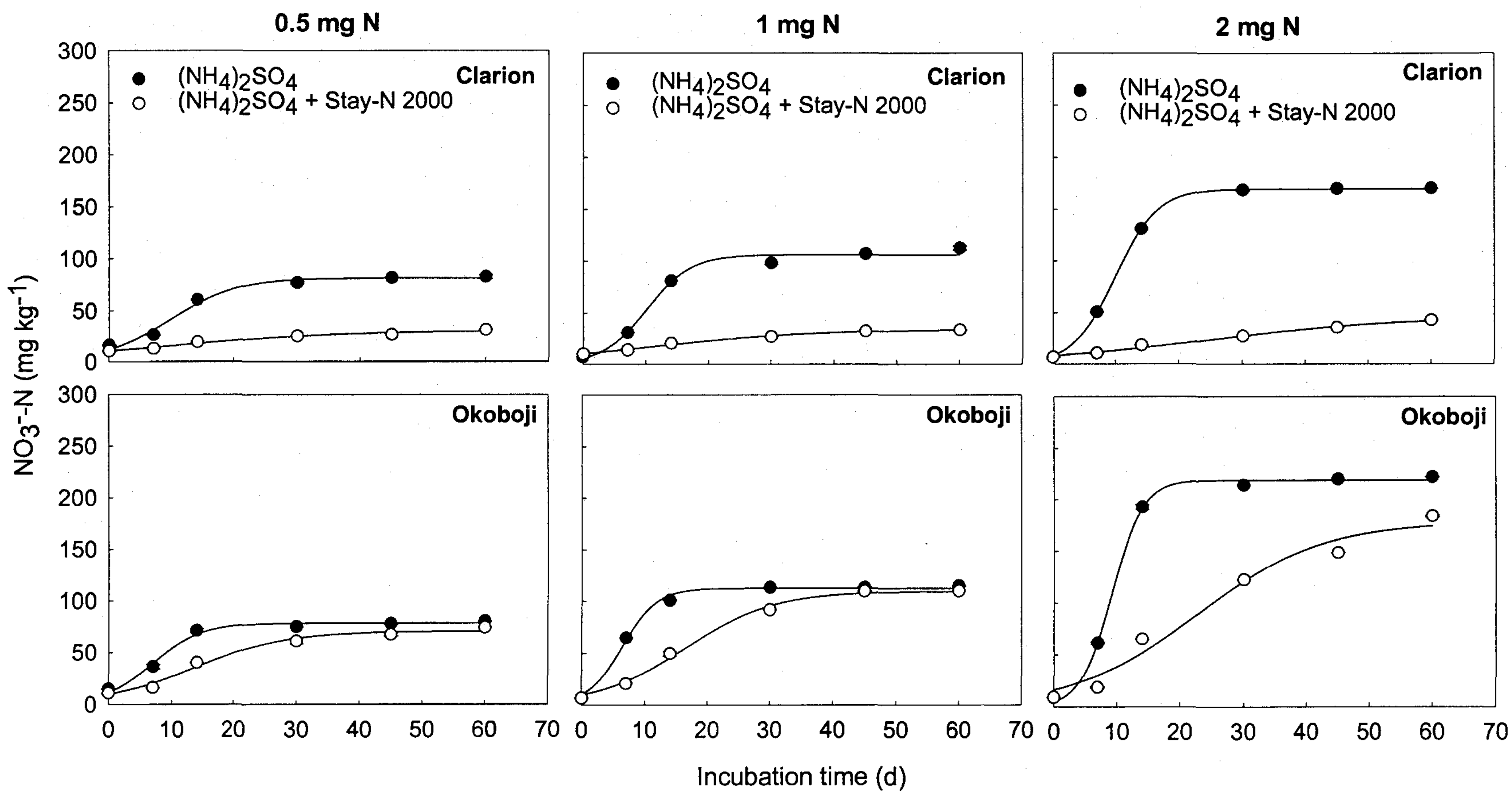

Figure 5. Nitrate-N accumulation in Iowa soils as affected by different rates of N applied.

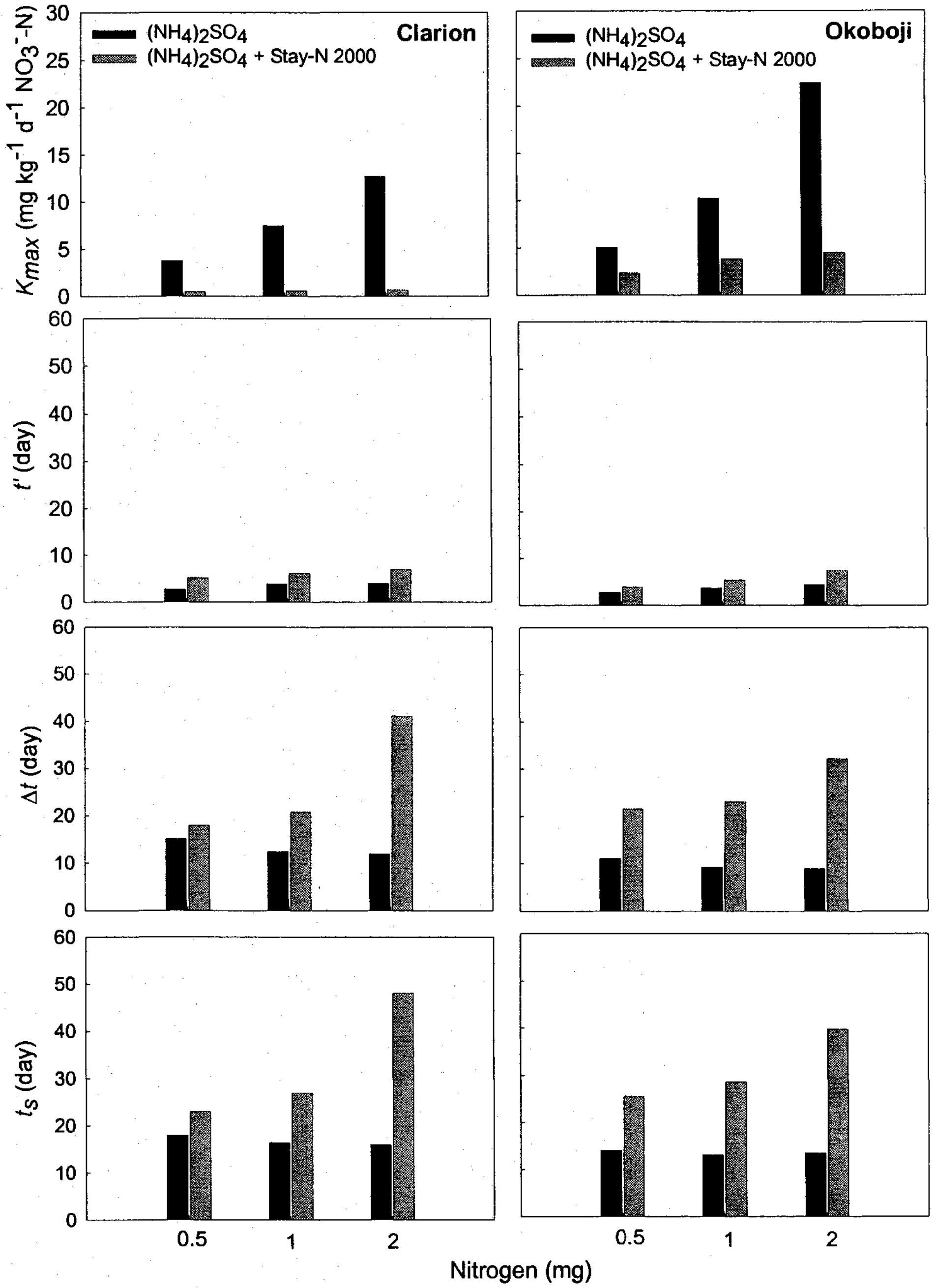

Figure 6. Calculated nitrification parameters of added $(NH_4)_2SO_4$ as affected by different rates of N.

CHAPTER 3. NITRIFICATION INHIBITION IN IOWA SOILS TREATED WITH STAY-N AS AFFECTED BY TEMPERATURE AND MATRIC POTENTIAL

A paper to be submitted to the Communication in Soil Science and Plant Analysis Journal

D. Rovita and R. Killorn

ABSTRACT

The effects of temperature and water potential on the rate of nitrification were investigated in two different cultivated soils varying markedly in C (OC), pH, and texture. Both soils were treated with 1 mL of solution containing 2 mg N $(NH_4)_2SO_4$, 4 µg a.i. g^{-1} soil of Stay-N 2000 (reformulated nitrapyrin) and additional deionized water to bring the soil water potential to -1, -10, and -60 kPa. All samples were incubated at 10, 20, and 30°C. After 0, 7, 14, 30, 45, and 60 days of incubation, soil was removed to determine the nitrification rates. To estimate the course of nitrification inhibition, a first-order equation was used to calculate the maximum nitrification rate (K_{max}), duration of lag period (t'), period of maximum nitrification (Δt), and duration of lag plus maximum nitrification period (t_s). The NO_3^--N accumulation increased with increasing temperature in all moisture levels in both soils and reduced when the soil was incubated at 10, 20, and 30°C, respectively, at soil moisture level of -1 kPa. Nicollet and Canisteo soils exhibited their maximum nitrification at different matric potentials. The highest maximum nitrification rate (K_{max}) occurred at 30°C and at a moisture potential level of -10 kPa (Fig. 4) in both control and treated soils of Nicollet and Canisteo. Addition of Stay-N 2000, however, reduced the K_{max} to 16 and 9 mg kg^{-1} d^{-1} NO_3^--N, respectively, in Nicollet and

Canisteo soils. The maximum rates varied greatly between the two soils and temperatures beyond this moisture level. The extension of *t'* due to the addition of Stay-N 2000 was as high as 7 d in Nicollet soil at 10°C and -1 kPa, and as little as 2 d in Canisteo soil at 20°C and - 10 kPa. The overall result from this study provided some insight into conditions that limit the nitrification process especially in association with applied nitrification inhibitor Stay-N 2000.

INTRODUCTION

In most agricultural soils, the ammonium (NH_4^+-N) form of fertilizer is quickly converted to nitrate (NO_3^--N) by the process of nitrification. Nitrate can be leached, taken up by plants or reduced and lost in gaseous forms. This process is crucial to the efficiency of N fertilizers and their impact on the environment. Reviews on nitrification include those of Painter (1970), Focht and Verstraete (1977), and Schmidt (1982).

In modern agro-ecosystems where large quantities of N fertilizers are used continuously in the form of NH_4^+-N or NH_4^+-producing compounds such as urea, NH_4^+-N is oxidized quite rapidly to nitrate (Puttanna et al., 2001). The efficiency of their use may be low and may result in environmental pollution due to loss by leaching and denitrification. Concern about pollution of ground and surface waters by fertilizer-derived nitrate has stimulated research to find compounds that will effectively inhibit nitrification of fertilizer N when applied to soils. The use of nitrification inhibitors may help conserve N fertilizers in situations where leaching or denitrification is a problem.

Numerous compounds have been patented or proposed for this purpose (Bundy and Bremner, 1973; McCarty & Bremner, 1989; McCarty, 1999; Puttanna et al., 1999). Nitrapyrin [*2-chloro-6-(tricholoromethyl)-pyridine*], manufactured by Dow Chemical Company, is the most widely evaluated nitrification inhibitor (Hauck, 1980; Walters and Malzer, 1990; Bronson *et al.*, 1992) and commercially available under the trade name of N-Serve. It has U.S. Environmental Protection Agency approval for use on cropland in the United States (Goring, 1962; Goos, 1985). N-serve is a volatile product and can be easily lost if not incorporated into the soil. In addition, N-serve needs an emulsifying agent in order to ensure complete dissolution. Recently, nitrapyrin has been reformulated and is currently being sold as Stay-N 2000, developed by Platte Chemical Co (Greeley, CO). Nitrapyrin has previously used xylene with a flash point of 79°F as its solvent and carrier while Stay-N 2000 uses an Exxon-Mobil solvent Aromatic 200 with a flash point of 200°F. The reformulated product is emulsifiable and less volatile than nitrapyrin.

Nitrification is performed by nitrifying organisms that obtain the energy for their metabolic functions from the oxidation of ammonium into nitrate in the process. The activities of these organisms are greatly influenced by several environmental factors. Among those factors that affect nitrification in soils are temperature and moisture (Stevenson, 1986).

It has been long known that soil temperature has an influence on the rate of nitrification (Tandon and Dhar, 1934) specifically, in retarding the production of NO_3^--N from NH_4^+-N added to the soil system. In addition, temperature is one of the main physical factors affecting the efficacy of nitrification inhibitors in soils as it affects both the volatility and rate of hydrolysis of the nitrification inhibitor when it is added together with N fertilizers (Herlihy and Quirke, 1975). The effect of moisture on soil nitrification process is substantial and subject to intense research as well. Robinson (1957) studied nitrification in tropical soils at soil moisture near wilting point, and Calder (1957) studied nitrification at soil moisture levels between wilting point and saturated conditions. Most other nitrification studies were performed at field capacity or values near 50% of saturation. The results suggested that maximum nitrification rates occur between -10 and -33 KPa (Haynes, 1986). Considerable research has been conducted to determine the effect of temperature and soil water potential which control the nitrification process in recent years (Justice and Smith, 1962; Miller and Johnston, 1964; Sabey, 1969; Malhi and McGill, 1982). However, not all combinations of moisture and temperature were included in most studies.

Understanding the kinetics of nitrification is allowing investigation of the interacting physical, chemical and biological factors involved in controlling the mechanism of this process in soils. The kinetics of nitrification has been studied in pure and mixed enrichment culture, and modeled in the soil environment (Prosser, 1989). As with many other biological reactions, the course of nitrification in soils is characterized by a sigmoidal curve with a delay period during which the number of bacteria increase, a maximum rate phase and a termination phase due to NH_4^+-N depletion (Sabey et al., 1959; Hadas et al., 1986). Several models have been used to describe nitrification rates in soil system, such as zero order kinetics (Sabey et al., 1969; Beek and Frissel, 1973; Addiscott, 1983), first order equations (Mehran and Tanji, 1974; Cameron and Kowalenko, 1976), and a microbiological approach based on Michaelis-Menten kinetics (Ardakani et al., 1974; van Veen and Frissel, 1981; Malhi and McGill, 1982). However, in some instances, it is difficult to use them for the prediction of the nitrification process in soils due to the complex interaction related to their microbiological parameters. Therefore, additional information is necessary to relate nitrification inhibition with time in a wide range of soils amended with N.

Since very few investigations included the combination of temperature and moisture affecting the nitrification process in soils, this study was undertaken to find the effect of temperature and soil moisture potentials on the efficacy of Stay-N 2000 in delaying the nitrification of applied NH_4^+-N in Iowa soils. This study was carried out by characterizing the nitrification process quantitatively using a nonlinear regression equation in order to be able to calculate the approximate kinetic parameters of maximum nitrification rate (K_{max}), duration of lag period (t'), period of maximum nitrification (Δt), and duration of lag plus maximum nitrification period (t_s).

MATERIALS AND METHODS

Soils

The soils used (Table 1) were surface (0-15 cm) samples representative of the Nicollet and Canisteo series collected in the summer 2002 from cultivated soils near Ames, Iowa. These soils were selected to obtain ranges in pH (5.8 - 8.2), OC (25.1 - 34.3 g kg^{-1}), total N (2.3 - 2.9 g kg^{-1}), and texture (258 - 277 g kg^{-1} clay and 341-355 g kg^{-1} sand). Each soil was air-dried and crushed to pass a 2 mm screen. In the analysis reported in Table 1, pH was determined with a glass electrode (soil:water or 0.01 M $CaCl_2$ ratio 1:2.5). Total C and N were determined by using a CHN-2000 Leco. Organic C was obtained by previously determining the $CaCO_3$ content using a Fluke 70 III multimeter in order to be able to calculate the inorganic C (%$CaCO_3$ x 0.1199), then the number is subtracted from the total C. Particle size distribution was determined by the pipette method of Kilmer and Alexander (1949).

Water potential is an expression of the physical forces holding water in soil. In this experiment three soil moisture potential levels (-1, -10, and -60 kPa) were applied as moisture treatments. These levels were obtained by using a tension table and pressure plates (Klute, 1986). Pressure potential was documented within a range of 0 to -75 cm by adjusting the height of water column. Soil samples were allowed to reach equilibrium and weighed to calculate the gravimetric water content. A pressure plate was used in order to determine the water content (g/g soil) at -33.3, -100, and -300 kPa. At this step, the chamber was depressurized after reaching equilibrium at each pressure, and soil samples were removed and weighed to calculate the mass of water.

Procedures

A soil sample (10 g) was placed in a 118-mL French square bottle and treated with 2 mL of solution containing 2 mg N as $(NH_4)_2SO_4$ and 4 μg a.i. g^{-1} soil of Stay-N 2000. Stay-N 2000 was obtained from Platte Chemical Co., Greeley, Colorado. Additional deionized water was added to bring the soil moisture content to -1, -10, or -60 kPa. All bottles were then covered with parafilm perforated in the center for aeration and placed in an incubator at 10, 20, and 30 ± 1°C and 90% r.h., for 0, 7, 15, 30, 45, and 60 d. The amount of water lost during incubation was replaced if the loss exceeded 1 g by measuring the water content of the sample gravimetrically every week.

At the end of incubation, each sample was extracted with 50 mL of 2 M KCl solution. The suspension was filtered through a Whatman no. 1 filter after equilibration for 1 h. The extract was analyzed for (NO_3^- + NO_2^-)-N and NH_4^+-N using a QuickChem AE Automated Ion Analyzer (Lachat Instruments, 1990).

The nitrification rate equations were calculated using the nonlinear procedure of SAS (SAS Institute, 2003).

Kinetic Parameters

The nonlinear regression of Hadas et al. (1986) was used to estimate the accumulation of NO_3^--N, which upon integration, gives a sigmoidal curve:

$$NO_3^- = a/\{1 + (a/[NO_3^-]_0 - 1)\exp(-ak[t - t_0])\} \qquad [1]$$

where NO_3^- is NO_3^- accumulation (mg NO_3^--N kg^{-1}) at a particular time, a is the asymptotic value of NO_3^- and $(NO_3^-)_0$ is the initial value of NO_3^- at time zero (t_0), k is a constant, and t_0 is the initial time, which equals zero. The maximum nitrification rate (K_{max}) was estimated by using the equation developed by Sabey et al. (1959) and Hadas et al. (1986) as the maximum slope of eq [1], at the inflection point (when NO_3^- = a/2):

$$K_{max} = k \times a^2/4 \qquad [2]$$

The duration of lag period (t') was calculated from the formula described by Sabey et al. (1959) and Hadas et al. (1986):

$$t' = (1/ak)\ln[a/(NO_3^-)_0 - 1] + [(NO_3^-)_0 - a/2]/K_{max} \quad [3]$$

The duration of period of maximum nitrification (Δt) was calculated by using the Laubscher et al. (1990) equation.

$$\Delta t = 2[a/2 - (NO_3^-)_0]/K_{max} \quad [4]$$

The termination period of the maximum nitrification rate (t_s), can be determined after obtaining the duration of the delay period (t') and the duration of the period of maximum nitrification (Δt) described by Laubscher et al. (1990).

$$t_s = t' + \Delta t \quad [5]$$

RESULT AND DISCUSSIONS

Incubation temperatures and soil water potentials in both Nicollet and Canisteo soils treated with Stay-N 2000 affected accumulation of NO_3^--N. We illustrate the accumulation of NO_3^- due to the soil temperature and matric potential using the first order kinetic equation described by Hadas et al. (1986). Incubation temperature and soil moisture level markedly altered the course of nitrification as indicated by the accumulation of NO_3^--N (Fig. 1, 2, and 3). The accumulation of NO_3^--N increased with increasing temperature at all moisture levels in both soils and when Stay-N 2000 was included, the accumulation of NO_3^--N reduced to 70, 64, and 62% in the Nicollet soil and 67, 60, and 54% in the Canisteo soil when the soil was incubated at 10, 20, and 30°C, respectively, at soil moisture level of -1 kPa. At a moisture level of -10 kPa, the reduction of NO_3^--N was 35, 64, and 48% in Nicollet soil; and 72, 63, and 6% in the Canisteo soil when the soil respectively at 10, 20, and 30°C. The highest accumulation of NO_3^--N was found in the Canisteo soil both in control and treated soil samples at 30°C and at all soil moisture levels. A moisture level of -60 kPa is the driest condition in our experiment. At this level, the highest accumulation NO_3^--N among control and treated soils was found in the Canisteo soil when the samples were incubated at 30°C. The Canisteo soil having higher organic C content compared to the Nicollet soil had the highest NO_3^--N accumulation. It is indicated by Bundy and Bremner (1973) and Lewis and Stefanson (1975) that the effectiveness of this compound decreases with increasing inorganic matter. Organic matter is known as major sorbing component of nitrapyrin (Hendrickson and Keeney, 1978). We believe that Stay-N 2000 posses some characteristics of nitrapyrin, therefore the mode of action of this compound is somewhat similar to nitrapyrin.

We employed equations 2, 3, 4, and 5 to calculate all kinetic parameters involved in our experiment. These equations were derived from the nonlinear regression described by Hadas et al. (1986) to obtain the k, a, and $(NO_3^-)_0$ values in order to be able to calculate the maximum nitrification rate (K_{max}), the lag period (t'), the maximum nitrification period (Δt), and the period of termination (t_s) in figures 1, 2, and 3. All curves show a lag period corresponding to a population growth of nitrifying bacteria (Morrill and Dawson, 1967), a maximum nitrification phase, and a termination phase due to NH_4^+ depletion (Laubscher et al., 1990).

The highest maximum nitrification rate (K_{max}) occurred at 30°C and at moisture potential level of -10 kPa (Fig. 4) in both control and treated soils of Nicollet and Canisteo soils. Addition of Stay-N 2000, however, reduced the K_{max} to 16 and 9 mg kg^{-1} d^{-1} NO_3^--N, in Nicollet and Canisteo soils, respectively. Beyond this moisture level, the maximum rate varied greatly between the two soils and temperatures. The lowest rate was found at 10°C and -1 kPa in the control Nicollet soil, which was 0.5 mg kg^{-1} d^{-1} NO_3^--N. The soil was the wettest condition among all soil moistures applied. These results suggest that nitrification occurred in saturated soils even though the rate is slower than a drier soil condition. Our results are consistent with Barraclough et al. (1985) who discovered that nitrification took place in a thin oxidized layer of flooded soils at the soil-water interface. At the moisture level of -60 kPa, however, the K_{max} values of both Nicollet and Canisteo soils were lower than those at -10 kPa. The results indicated that the K_{max} was 11 and 19 mg kg^{-1} d^{-1} NO_3^--N in Nicollet and Canisteo soils, respectively; and decreased to 9 and 13 mg kg^{-1} d^{-1} NO_3^--N when Stay-N 2000 was applied. From all K_{max} values in this study, appreciable nitrification rates can be expected at 60% water holding capacity or -10 kPa, and Linn and Doran (1984), and Paul and Clark, (1996) found that the rates of microbial processes are generally most rapid near field capacity, and decline as soil matric potential becomes more negative. The temperature is one important factor affecting microbial activity in soils (Parton et al., 1987; Insam, 1990). Metabolic activities of microorganisms respond to temperature changes, with $Q_{10} \approx 2$ (Kilham, 1994). On the other hand, moisture potential also has substantial affect on microbial activities. Griffin (1981) concluded that the direct effect of soil moisture potential upon microbial activity is important. At higher moisture potentials the NH_4^+ substrate limitation, which limits microbial activity was reported as the rate-limiting factor (Skopp et al., 1990).

The lag period (t') of nitrification also varied between the two soils and experimental conditions. Maximum nitrification rates (K_{max}) increased due to increasing in temperatures, which generally decreased the t' (Fig. 4 and 5). The magnitude of the influence of temperature on the actual K_{max} and t', however, was different among soils (Frederick, 1957). The results from our experiment indicate that the longest extension of t' was found in Nicollet soil at -1 kPa and at 10°C and this decreased with increasing temperature. At the same soil moisture potential, the extension of t' in the Canisteo soil was higher than at the two other levels of moisture potential applied in our experiment. Overall, the extension of t' due to the addition of Stay-N 2000 was as high as 7 d in the Nicollet soil at 10°C and -1 kPa, and as little as 2 d in the Canisteo soil at 20°C and - 10 kPa (Fig. 4). The variation of lag period is probably due to the two most influential factors governing the lag period in this experiment, temperature and moisture potential. Stark and Firestone (1995) confirmed that cell dehydration became rate limiting for nitrification when the moisture potentials become more negative and at warmer temperature. This finding was attributed to the decreased diffusion of soluble substrate into microbial cells. The maximum rate of nitrification (Δt) of control soils proceeded for 39.3 d in Nicollet soil followed by 27.9 d in Canisteo soil at -1 kPa and 10°C. The shortest Δt was 10 d in Canisteo soil at -60 kPa and 30°C (Fig. 6). As was expected, t_s followed the same trend as t' and Δt.

Soil moisture matric potential alters rates of nitrification at warmer soil temperatures especially when warmer temperatures are accompanied by more negative matric potentials. In addition, both the Nicollet and Canisteo soils exhibited a different rate of maximum nitrification with different temperatures and matric potential. The soil type seemed to play an important role in determining the degree of Stay-N 2000 inhibition as affected by temperature and moisture potential.

SUMMARY AND CONCLUSIONS

From this experiment, we assumed that maximum rates of nitrification of soils treated with Stay-N 2000 at 30°C are limited by diffusion of substrate to metabolically active cells of nitrifying bacteria involved in the process. At 20°C, we also assumed that substrate demand was not as high as at 30°C, therefore, the limitation of substrate diffusion is lessened. At 10°C on the other hand, the nitrification rates were the least among three temperatures levels applied.

Nitrification rates in this experiment were strongly influenced by soil moisture. It increased as soils go from more negative water potential to about field moisture capacity and declined with increasing water content where we assumed that O_2 becomes limiting. Nicollet and Canisteo soils exhibited their maximum nitrification at different matric potentials.

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Table 1. Initial properties of soil used

	pH		Organic	Total	Inorganic N			
Soil series	H_2O	$CaCl_2$	C	N	NH_4-N	NO_3-N	Clay	Sand
			----g kg^{-1} soil---		--mg kg^{-1} soil--		------g kg^{-1}------	
Nicollet	5.8	5.4	25.1	2.3	7	18	258	341
Canisteo	8.2	7.6	34.3	2.9	8	7	277	355

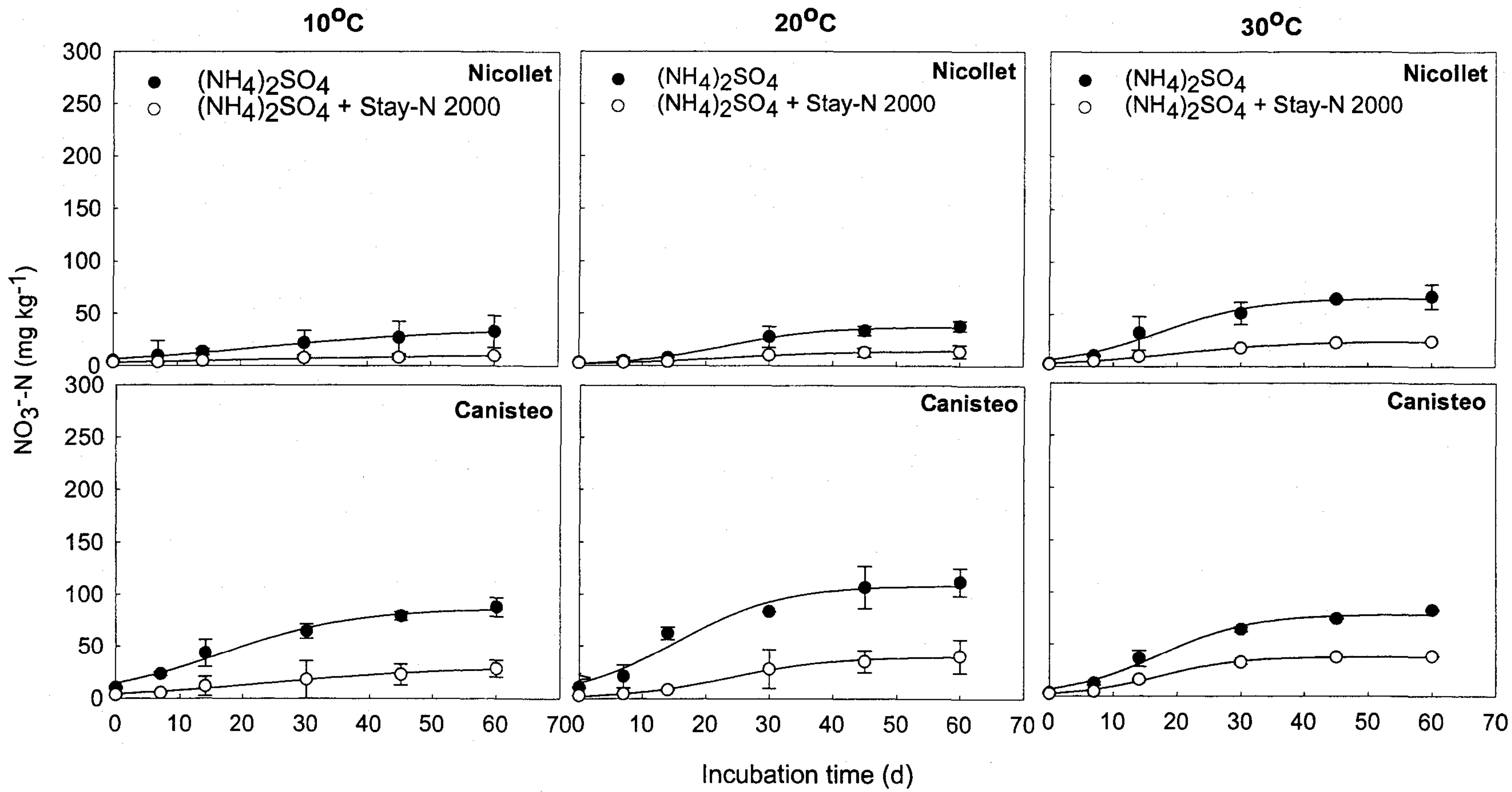

Figure 1. Nitrate-N accumulation in Iowa soils incubated at different temperatures at -1 kPa matric potential.

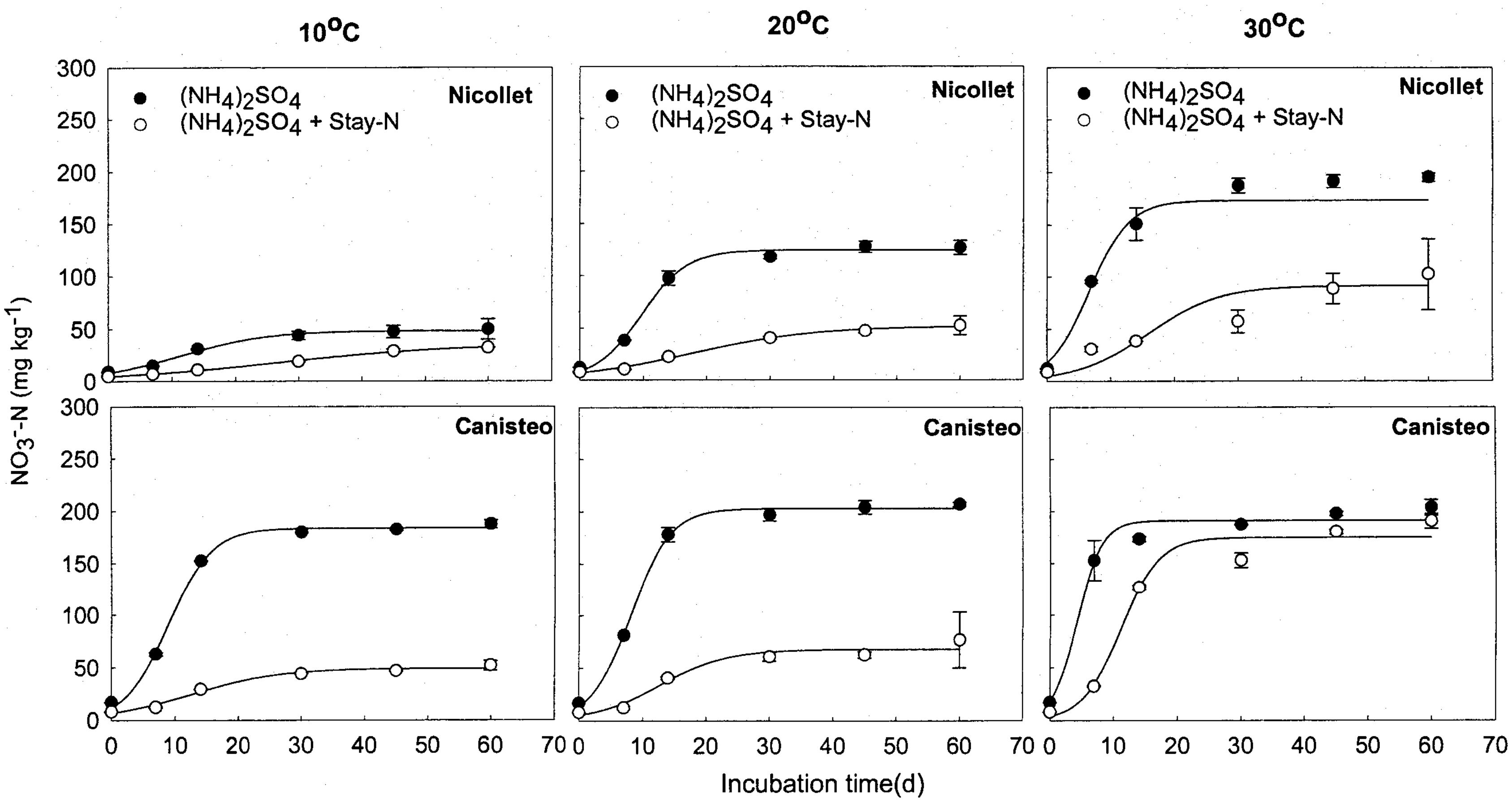

Figure 2. Nitrate-N accumulation in Iowa soils incubated at different temperatures at -10 kPa matric potential.

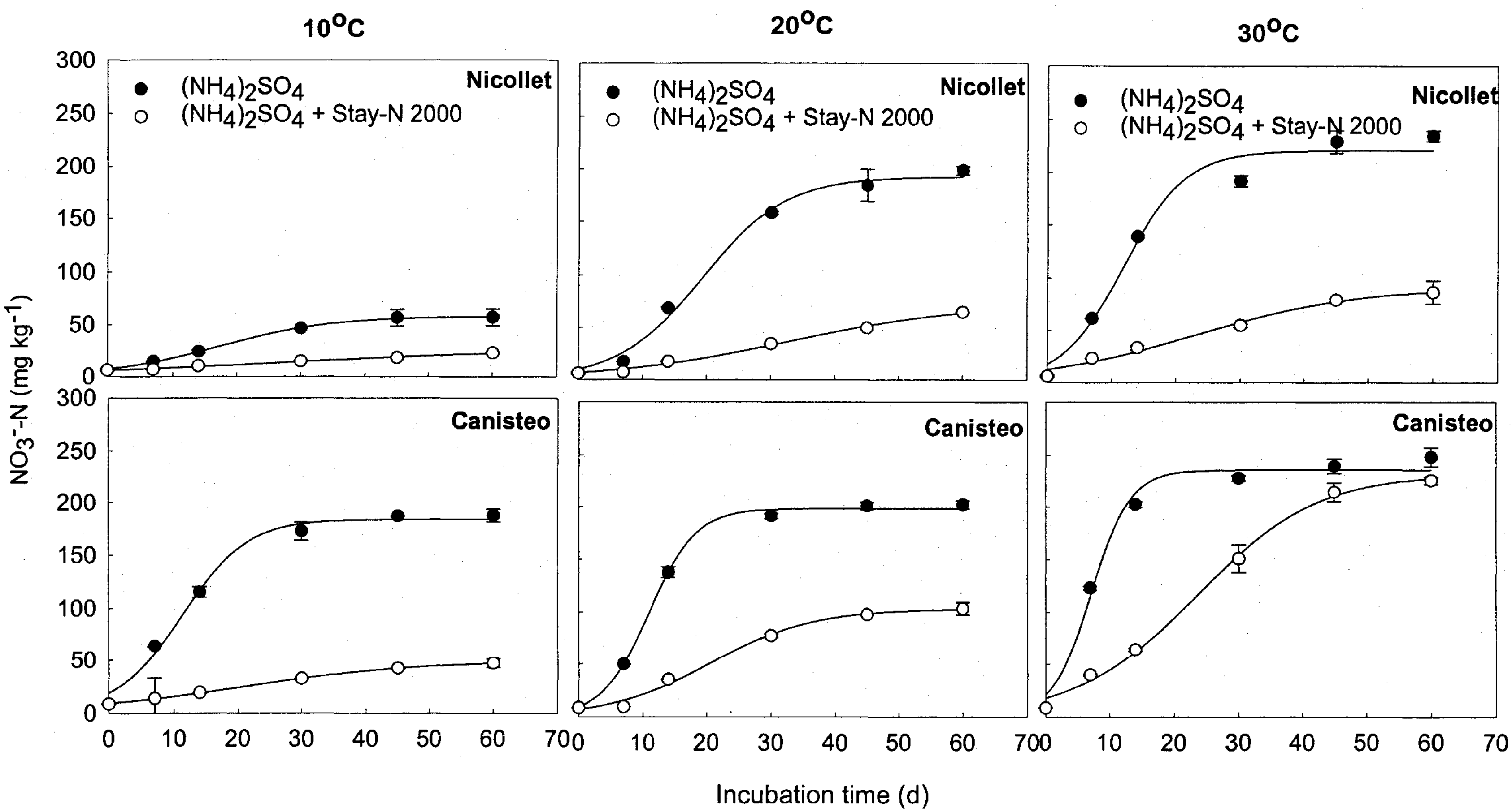

Figure 3. Nitrate-N accumulation in Iowa soils incubated at different temperatures at -60 kPa matric potential.

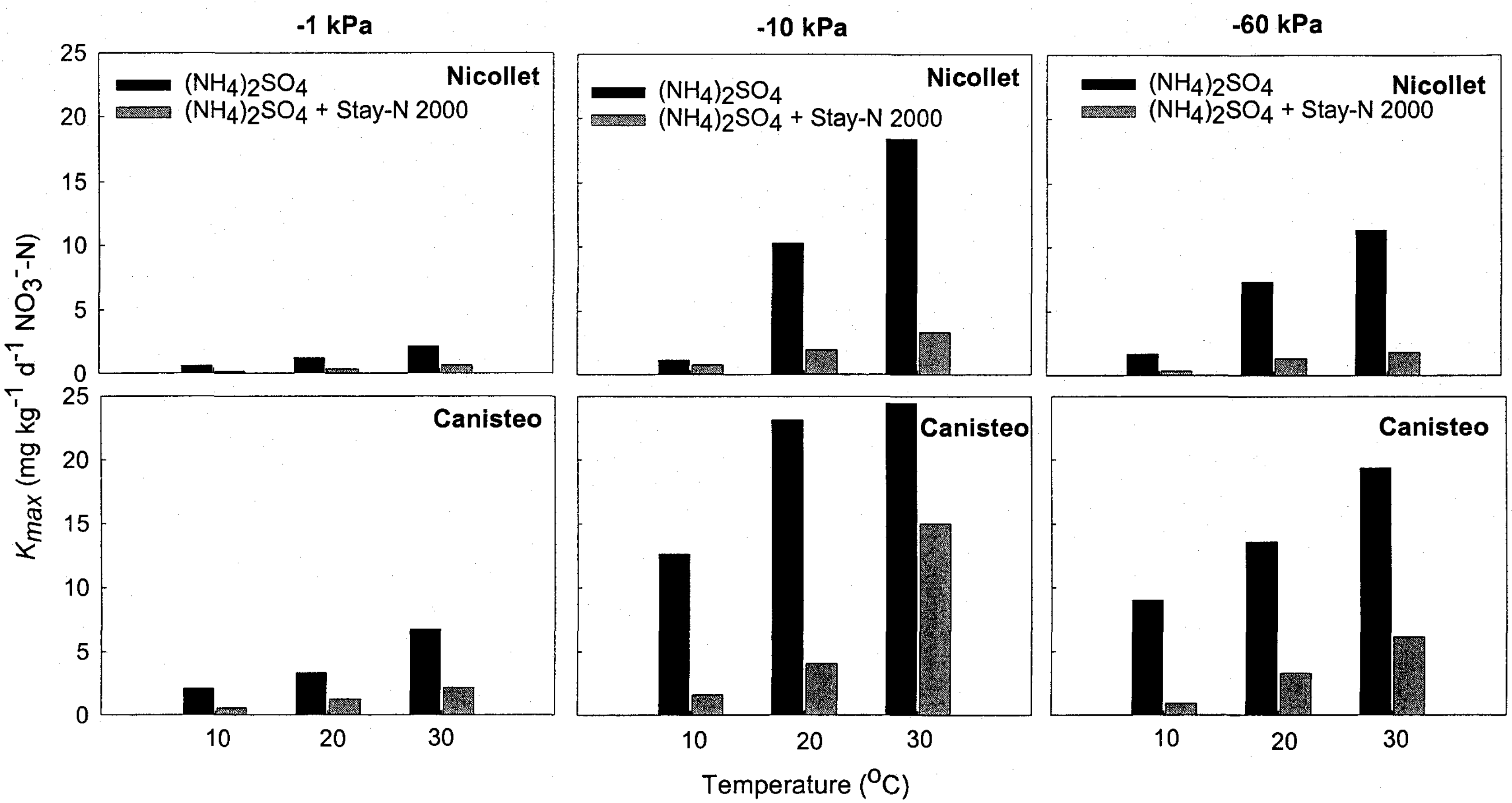

Figure 4. Maximum rates of nitrification (K_{max}) in Iowa soils as affected by temperatures and matric potentials.

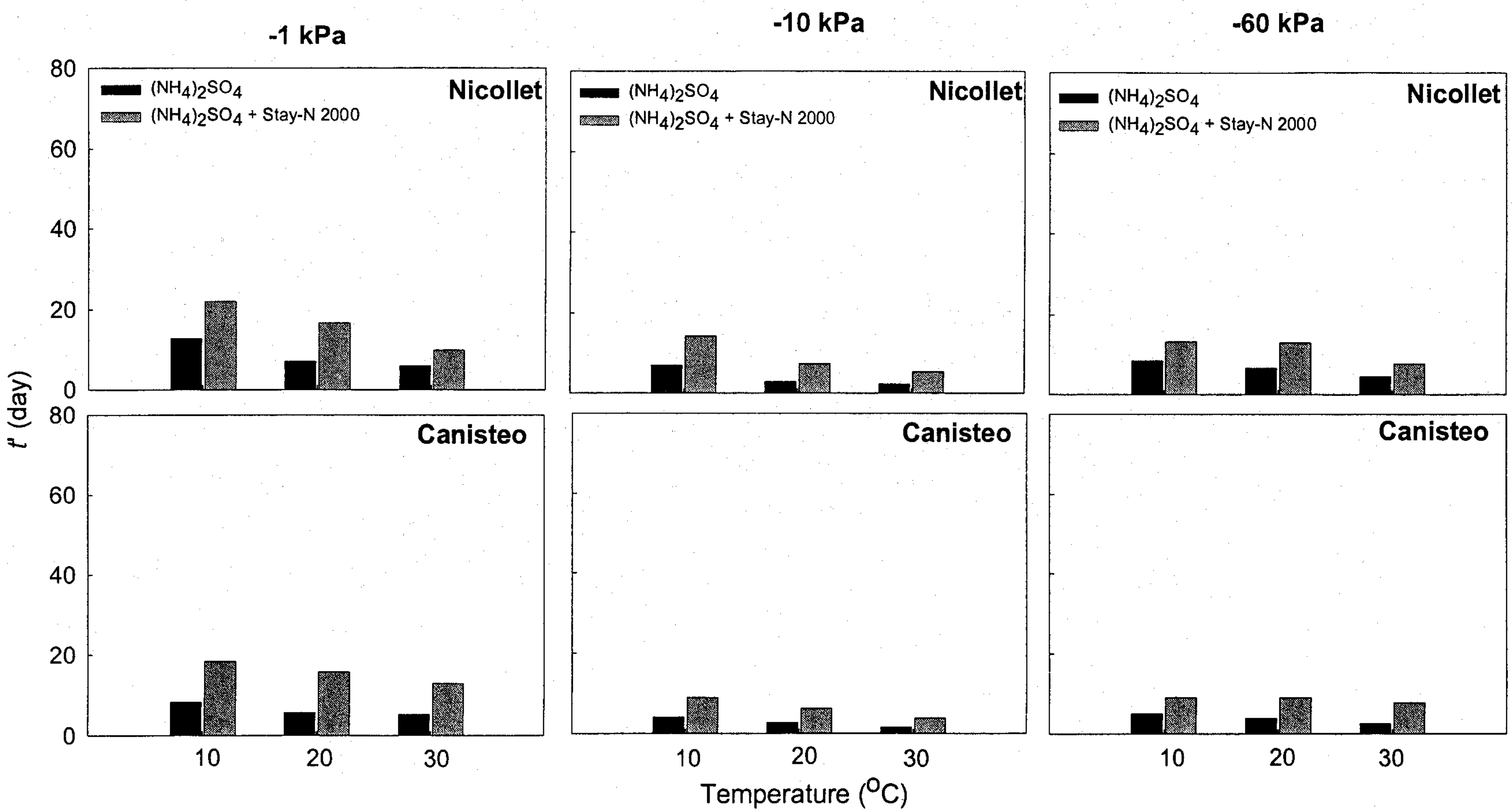

Figure 5. Delay period of nitrification (*t'*) as affected by temperatures and matric potentials.

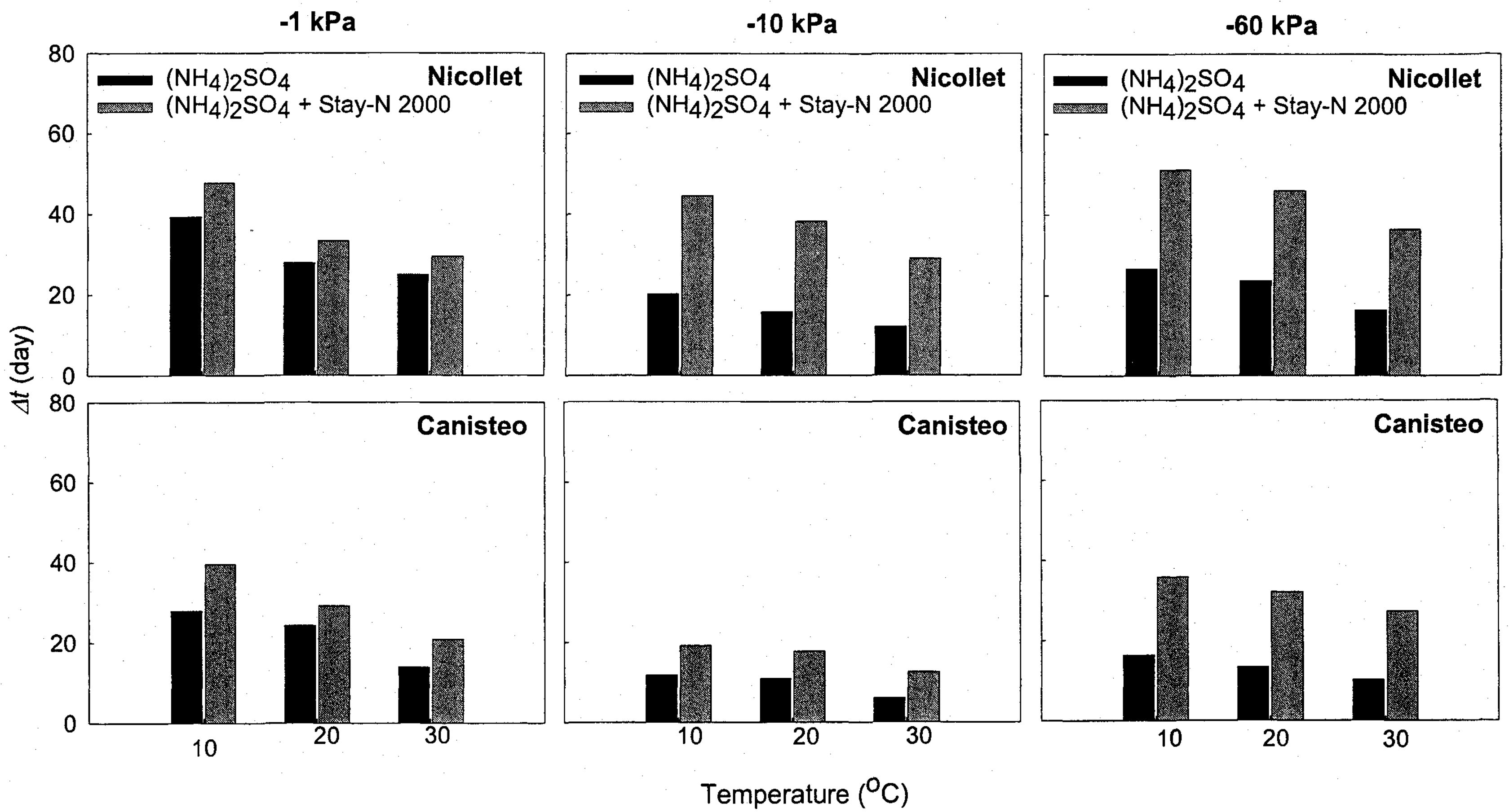

Figure 6. Period of maximum nitrification (Δt) in Iowa soils as affected by temperatures and matric potentials.

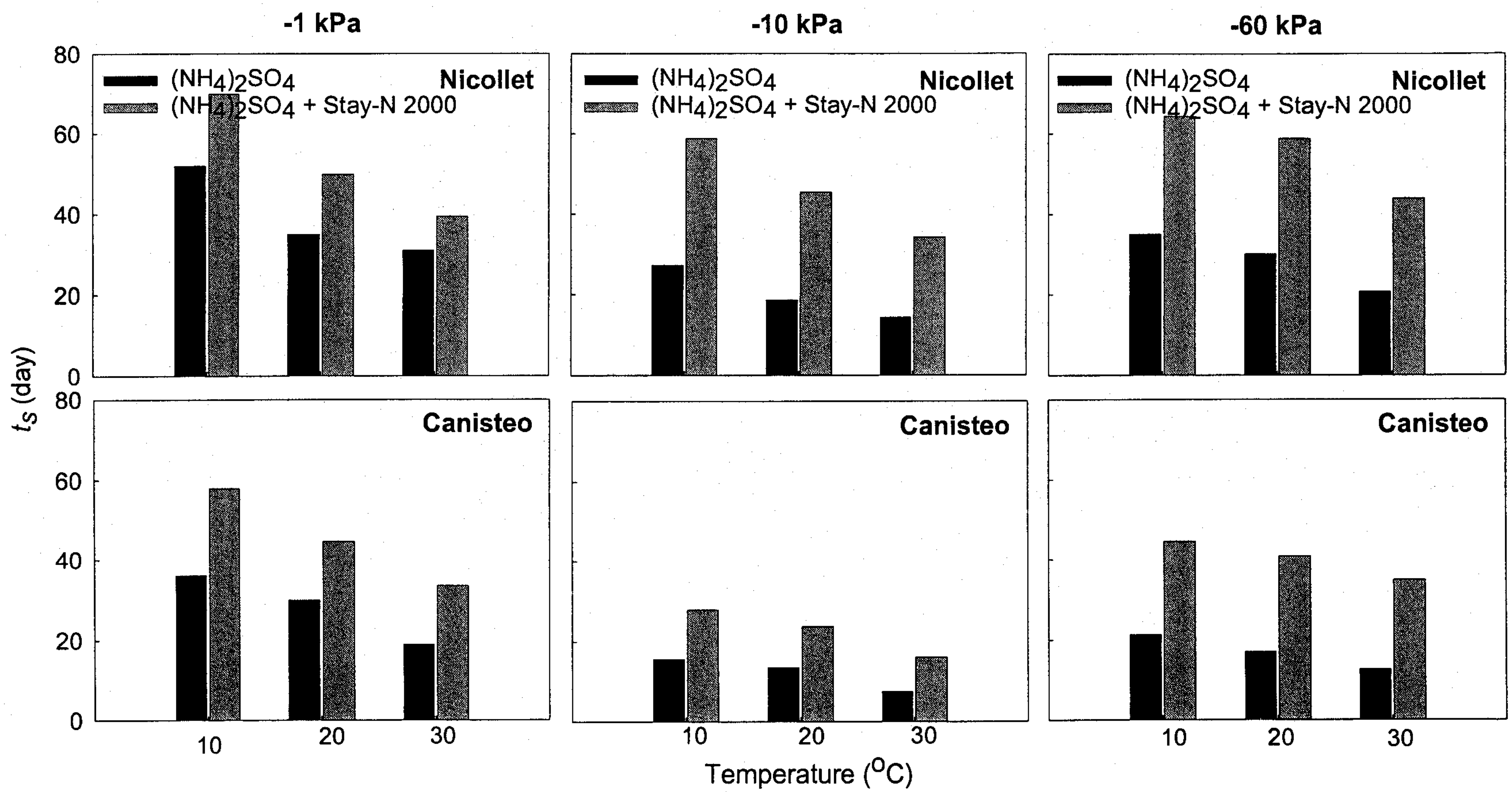

Figure 7. Termination of nitrification (t_s) in Iowa soils as affected by temperatures and matric potentials.

CHAPTER 4. THE EFFECT OF HEAVY METALS ON THE INHIBITION OF NITRIFICATION BY STAY-N 2000 IN IOWA SOILS

A paper to be submitted to the Journal of Environmental Quality

D. Rovita and R. Killorn

ABSTRACT

The nitrification process in soils is sensitive to some heavy metals. A laboratory experiment to study the effect of different heavy metals on nitrification rates in two Iowa soils varying markedly in organic C (OC), pH, and texture was conducted. Both soils were treated with $(NH_4)_2SO_4$ as a source of N followed by addition of Stay-N 2000 (reformulated nitrapyrin) as a nitrification inhibitor. To evaluate the effect of heavy metals on the effectiveness of Stay-N 2000 in inhibiting nitrification in both soils, Cu(II), Zn(II), Cd(II) as sulfates, and Pb(II) as the acetate were added to each soil. All soils were incubated at 20±1°C for 0, 7, 14, 30, 45, and 60 d. To estimate the course of nitrification inhibition, a first-order equation was used to calculate the maximum nitrification rate (K_{max}), duration of lag period (t'), period of maximum nitrification (Δt), and duration of lag plus maximum nitrification period (t_s). The K_{max} and the t' were affected by heavy metals when the soils were amended with Stay-N 2000. All metals used in this study seemed to reduce K_{max} in both the Clarion and Okoboji soils. In the Clarion soil (24.0 g kg^{-1} OC), the K_{max} of $(NH_4)_2SO_4$ was 12±1 mg kg^{-1} d^{-1} when the soil was without Stay-N 2000 but decreased to 4, 0.25, 0.86, and 0.27 mg kg^{-1} d^{-1} when a combination of Stay-N 2000 and Cu, Zn, Pb or Cd were added, respectively. The K_{max} of $(NH_4)_2SO_4$ in the Okoboji soil was 22±2 mg kg^{-1} d^{-1} in the soil without the addition of Stay-N 2000 but

reduced to 6, 3, 4, and 2 mg kg^{-1} d^{-1} when combination of Stay-N and Cu, Zn, Pb or Cd were added, respectively. The t' varied from 8 to 25 d in the Clarion soil and from 5 to 25 d in the Okoboji soil due to addition of Cu, Zn, Pb or Cd and Stay-N 2000. The influence of metals added with Stay-N 2000 on the K_{max} and the t' varied among soils indicating that other factors inherent in the soils also affect nitrification.

INTRODUCTION

Increases in production costs for N fertilizers have encouraged crop growers to seek less expensive sources of nutrients such as sewage sludge, and industrial, municipal and animal wastes (Kinkle et al., 1986). While there are positive crop responses to land application of these nutrient-rich compounds, possible negative effects this practice might have on soils must be considered because heavy metals, such as Cu, Zn, Pb, and Cd may be present as components (Garcia et al., 1991; Alloway, 1995)

There is concern about the effect of heavy metals at high concentration in the environment as they can persist for a long period of time, become irreversibly immobilized within different soil components (Corey et al., 1988), and affect some biochemical reactions in the biosphere. One example of a soil process that could be affected by heavy metals is nitrification (Premi and Cornfield, 1969; Wilson, 1977; Bewley and Stotzky, 1983; Dusek, 1995). Because of its sensitivity to metals and its key role in nitrogen cycling, the relative effect of heavy metals such as Cu, Zn, Pb, and Cd on the inhibition of nitrification in soils deserves investigation particularly when the soil is treated with a nitrification inhibitor.

Nitrification in soils is the microbial oxidation of NH_4^+-N via various intermediate stages to NO_2^--N, which is quickly oxidized to become NO_3^--N in a downstream reaction step. The NO_3^--N formed through this process is susceptible to loss by leaching, runoff, and denitrification that can lead to a reduction the efficiency of nutrients applied. Large losses of N through the above processes have long been of concern to soil scientists as they may contribute to NO_3^--N pollution of ground and surface waters.

Minimizing nitrogen losses resulting from application nutrients to the soil has been the subject of scientific work, both in agricultural research and in the fertilizer industry. In attempts to retard such losses, inhibitors of nitrification such as nitrapyrin [2-*chloro*-6-(*tricholoromethyl)-pyridine*] (Walters and Malzer, 1990; Bronson *et al.*, 1992) are employed. Nitrapyrin has U.S. Environmental Protection Agency approval for use on cropland in the United States (Goring, 1962; Goos, 1985) and is commercially available under the trade name of N-Serve. N-serve is a volatile product and this compound needs an emulsifying agent as well in order to ensure complete dissolution. Nitrapyrin uses xylene with a flash point of 79°F as its solvent and carrier. A number of laboratory and field trials have shown that nitrapyrin markedly reduced the rate of NO_3^--N formation following the addition of NH_4^+-N or NH_4^+-producing compounds (Goring, 1962; Touchton et al., 1978; and Owens, 1981). Recently, nitrapyrin has been reformulated and is currently being sold as Stay-N 2000, developed by Platte Chemical Co (Greeley, CO). It uses an Exxon-Mobil solvent Aromatic 200 with the flash point of 200°F, which is less volatile than nitrapyrin; and this compound is also emulsifiable.

The kinetics of nitrification has been studied and modeled in the soil environment (Prosser, 1989). Several models have been used to describe nitrification rates in the soil system, such as, zero order kinetics (Sabey et al., 1969; Beek and Frissel, 1973; Addiscott, 1983), first order equations (Mehran and Tanji, 1974; Cameron and Kowalenko, 1976), and a microbiological approach based on Michaelis-Menten kinetics (Ardakani et al., 1974; van Veen and Frissel, 1981; Malhi and McGill, 1982). However, it is still difficult to use these models for prediction of the nitrification process in soils, because some necessary information related to microbiological parameters are not included in the models (Hadas, et al., 1986). Therefore, additional information is required to relate the nitrification inhibition by nitrification inhibitors with time in a wide range of soils.

Because there are a limited number of studies evaluating the relative effects of metal containing fertilizer sources on nitrification of soils treated with inhibitors (Lagerwerff, 1972; Page, 1974), this study was conducted to determine the effect of heavy metals on the inhibition of nitrification in soils treated with Stay-N 2000 by comparing the changes the kinetic parameters of nitrification rate (K_{max}), duration of lag period (t'), period of maximum nitrification (Δt), and duration of lag plus maximum nitrification period (t_s).

MATERIALS AND METHODS

Soils

The soils used (Table 1) were surface (0-15 cm) samples representative of the Clarion and Okoboji soil series collected in the summer 2002 from cultivated soils near Ames, Iowa. These soils were selected to obtain ranges in pH (6.2 - 8.1), OC (23.6 - 41.7 g kg^{-1}), total N (1.9 - 3.5 g kg^{-1}), and texture (235 - 327 g kg^{-1} clay and 266 - 404 g kg^{-1} sand). Each soil was air-dried and crushed to pass a 2 mm screen. In the analysis reported in Table 1, pH was determined with a glass electrode (soil:water or 0.01 M $CaCl_2$ ratio 1:2.5). Total C and N were determined by using a CHN-2000 Leco. Organic C was obtained by determining the $CaCO_3$ content using a Fluke 70 III multimeter in order to be able to calculate the inorganic C (%$CaCO_3$ x 0.1199), then the number is subtracted from the total C. Particle size distribution was determined by the pipette method of Kilmer and Alexander (1949).

The heavy metals used were Fisher certified reagent-grade chemicals. Of these, Cu(II), Cd(II), and Zn(II) were added as sulfate and Pb(II) as acetate.

Procedures

A soil sample (10 g) was placed in a 118-mL French square bottle and treated with 3 mL of solution containing 50 µmoles of heavy metal, 2 mg N as $(NH_4)_2SO_4$, and 4 µg a.i. g^{-1} soil of Stay-N 2000. Stay-N 2000 was obtained from Platte Chemical Co., Greeley, Colorado. Additional deionized water was added to bring the soil moisture content to 60% of the water holding capacity. All bottles were then covered with parafilm perforated in the center for aeration and placed in an incubator at 20 $\pm$ 1°C and 90% r.h., for 0, 7, 15, 30, 45, and 60 d. The amount of water lost during incubation was replaced if the loss exceeded 1 g by measuring the water content of the sample gravimetrically every week.

At the end of incubation, each sample was extracted with 50 mL of 2 M KCl solution. The suspension was filtered through a Whatman no. 1 filter after equilibration for 1 h. The extract was analyzed for $(NO_3^- + NO_2^-)$-N and NH_4^+-N using a QuickChem AE Automated Ion Analyzer (Lachat Instruments, 1990).

The nitrification rate equations were calculated using the nonlinear procedure of SAS (SAS Institute, 2003)

Kinetic Parameters

This study was carried out by characterizing the nitrification process quantitatively using a non-linear regression of a sigmoidal curve obtained from NO_3^--N accumulation due to NH_4^+-N depletion in the process. The curve describes approximate kinetic parameters of maximum nitrification rate (K_{max}), duration of lag period (t'), period of maximum nitrification (Δt), and duration of lag plus maximum nitrification period (t_s) (Sabey et al., 1959; Hadas et al., 1986). Understanding kinetics of nitrification is allowing investigation of the interacting physical, chemical and biological factors involved in controlling the mechanism of this process in soils

The nonlinear regression of Hadas et al. (1986) was used to estimate the accumulation of NO_3^--N, which upon integration gives a sigmoidal curve:

$$NO_3^- = a/\{1 + (a/[NO_3^-]_0 - 1)\exp(-ak[t - t_0])\} \qquad [1]$$

where NO_3^- is NO_3^- accumulation (mg NO_3^--N kg^{-1}) at a particular time, a is the asymptotic value of NO_3^- and $(NO_3^-)_0$ is the initial value of NO_3^- at time zero (t_0), k is a constant, and t_0 is the initial time, which equals zero. The maximum nitrification rate (K_{max}) was estimated by using the equation developed by Sabey et al. (1959) and Hadas et al. (1986) as the maximum slope of eq [1], at the inflection point (when NO_3^- = a/2):

$$K_{max} = k(a^2/4) \quad [2]$$

The duration of delay period (t') was calculated from the formula described by Sabey et al. (1959) and Hadas et al. (1986):

$$t' = (1/ak)\ln[a/(NO_3^-)_0 - 1] + [(NO_3^-)_0 - a/2]/ K_{max} \quad [3]$$

The duration of period of maximum nitrification (Δt) was calculated by using the Laubscher et al. (1990) equation.

$$\Delta t = 2[a/2 - (NO_3^-)_0]/ K_{max} \quad [4]$$

The termination period of maximum nitrification rate (t_s), can be determined after obtaining the duration of delay period (t') and the duration of period of maximum nitrification (Δt) as it is described by Laubscher et al. (1990) equation.

$$t_s = t' + \Delta t \quad [5]$$

RESULTS AND DISCUSSIONS

Many potential nitrification inhibitors have been tested in soils or pure culture. However, studies regarding the precise mode of action of inhibitors have been neglected. It is understood that the enzyme ammonium monooxygenase, which catalyzes the oxidation of NH_4^+-N to NO_3^--N and has a constituent metal group, is one target for nitrification inhibitors (Hooper, 1978).

The results from our studies indicate that accumulation of NO_3^--N was affected by application of Stay-N 2000 and heavy metals in both soils (Fig. 1). The curves showed the lag period (t'), a maximum period of nitrification (Δt) and termination period of nitrification (t_s). The variation in inhibition varied between soil types and metals applied. The estimated asymptotic value parameter, *a*, was between 180 to 205 mg kg^{-1} in Clarion and Okoboji soils, respectively, when heavy metals were added. Application of Zn or Cd to both soils treated with Stay-N 2000 seemed to inhibit nitrification compared to Cu or Pb. All of the heavy metals applied to both soils in this experiment reduced the NO_3^--N accumulation and the K_{max}, t', Δt, and t_s compared to no inhibitor. Nevertheless, the standard errors of the means of calculated were small (Fig. 1) which indicated that the model fit nitrification process with its three phases in our laboratory studies.

When combined with Stay-N 2000, the addition of Cu, Zn, Pb or Cd to the Clarion soil reduced the K_{max} from 15 mg kg^{-1} d^{-1} to 4; 0.25; 0.86; and 0.27 mg kg^{-1} d^{-1}, respectively; and from 27 mg kg^{-1} d^{-1} to 6, 3, 4, and 3 mg kg^{-1} d^{-1}, respectively, in the Okoboji soil. Although the pattern of the inhibition is similar in both soils, the Okoboji soil with high pH and organic C showed less nitrification inhibition compared to Clarion soil. Hendrickson and Keeney (1979) studied the effectiveness of nitrapyrin and found that nitrifiers activity seemed to recover rapidly above pH 7.4 when the relative inhibition of nitrapyin declined due to the biodegradation of this compound. Studies by Goring (1962) found that the effectiveness nitrification inhibitors decreased with increasing organic C content and high pH. This is because the organic matter is the major sorbing component of nitrification inhibitors.

Copper was capable of reducing K_{max} in both soils, however, soils treated with Cu in the presence of Stay-N 2000, seemed to have a higher K_{max} compared to those soils treated with Zn, Pb, or Cd. This phenomenon is probably due to the fact that Cu, a component of cytochrome oxidase (Campbell and Aleem, 1965), was involved in the oxidation of NH_4^+-N into NO_3^--N (Hoffman and Lees, 1953). Most of the potential nitrification inhibitors have chelating properties to mediate inhibition by chelating metal group ammonium-oxidizing enzymes such as Cu (Lees, 1952; Hooper and Terry, 1973). We assume in this experiment that Cu decrease the effectiveness of Stay-N 2000 in inhibiting the copper enzyme and to reduce the nitrate accumulation and K_{max} below the level of that without addition of Cu (Fig. 1 and 2). There are variable results in many investigations with respect to Cu. Although we were using Stay-N 2000, a reformulated nitrapyrin in our experiment, results from our study are consistent with previous studies in liquid batch culture monitoring the growth of *Nitrosomonas europea* using nitrapyrin by Campbell and Aleem (1965) who found that addition of Cu reversed the inhibition of nitrification. Lipman and Burgess (1914) also found that Cu markedly stimulated nitrification. Studies by Lipman and Burgess (1917), however, showed that addition of Cu increased the inhibition of nitrification. Although Campbell and Aleem (1965) did not mention the sources of Cu they used in their studies, these conflicting results may be due to the use of different sources of Cu or different strains of bacteria. Different form of heavy metals can have different effects on microbial physiology (Babich and Stotzky, 1980). Other differences also can exist in the experimental methods used by investigators. Compared to Cu, application of Zn or Cd increased the effectiveness of Stay-N 2000 in inhibiting nitrification, while application of Pb has no effect on the nitrification inhibition in both the Clarion and Okoboji soils (Fig. 1).

The addition of metals extended the lag period (t'). The t' varied from 8 to 25 d in the Clarion soil and from 5 to 9 d in the Okoboji soil due to addition of Cu, Zn, Pb or Cd and Stay-N 2000. When the maximum rate of nitrification decreased, the delay period increased in both soils (Fig. 2). The addition of Zn, Pb, and Cd to both soils extended the period of maximum nitrification (Δt) to 97, 37, and 43 d respectively in Clarion soil and to 38, 26 and 32 d, respectively in Okoboji soil. In addition, the Pb effect on nitrification in this experiment is not as detrimental as Zn and Cd as indicated by all parameters. Irving and Williams (1948) stated that Pb is a more electronegative element than Zn and forms more stable chelates and less soluble complexes in the soil system than does Zn. This could explain the relative low inhibition by Pb compared to Zn. No information was available on the complexes of Cd in the soil. The termination phase (t_s) also increased with the addition of Stay-N 2000 together with heavy metals. Accordingly, the soil type seemed to play an important role in determining the degree of Stay-N 2000 inhibition on nitrification when it is accompanied by heavy metals.

SUMMARY AND CONCLUSIONS

The results of this experiment indicate that the application of heavy metals to the soils treated with Stay-N 2000 affected the inhibition of nitrification in both the Clarion and Okoboji soils. The nitrification inhibition in both soils studied varied among heavy metals applied. Application of heavy metals decreased the maximum rate of nitrification (K_{max}), extended the delay period (t') as well as the maximum period of nitrification (Δt) in both soils compared to no nitrification inhibitor and no metals added. Stay-N 2000 was less effective in reducing the nitrate accumulation when applied with Cu. Our results showed that the level of NO_3^--N accumulation in the soil treated with Cu in the presence of Stay-N 2000 was above that of Stay-N alone. Copper is one component of ammonium oxidizing enzymes. We assumed that the chelating property of Stay-N 2000 in chelating metal groups of ammonium-oxidizing enzymes is different from other nitrification inhibitors. Stay-N 2000 also seems to have the same mode of action when it is applied with different metals as can be explained from the kinetic parameters in this study. The results obtained for Pb differ from those obtained for Zn and Cd. Combination of Stay-N 2000 and Pb seemed to have the same inhibitory effect on nitrification as Stay-N 2000 alone. On the other hand, when Stay-N 2000 was combined with Zn or Cd, each of these metals increased the effectiveness of Stay-N 2000 in inhibiting the nitrification in both the Clarion and Okoboji soils. Based on the NO_3^--N accumulation, the inhibitory ranking of the four tested heavy metals with inhibitors appeared as follows: Zn>Cd>Pb>Cu.

The soil type seemed to play an important role in determining the degree of Stay-N 2000 inhibition of nitrification when it is accompanied by heavy metals. The overall result from this study provided some insight into conditions that limit the nitrification process especially in association with applied nitrification inhibitor Stay-N 2000.

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Table 1. Initial properties of soil used

	pH		Organic	Total	Inorganic N			
Soil series	H_2O	$CaCl_2$	C	N	NH_4-N	NO_3-N	Clay	Sand
			----g kg^{-1} soil---		--mg kg^{-1} soil--		------g kg^{-1}------	
Clarion	6.2	5.8	23.6	1.9	2	4	235	404
Okoboji	8.1	7.6	42.7	3.5	7	6	327	266

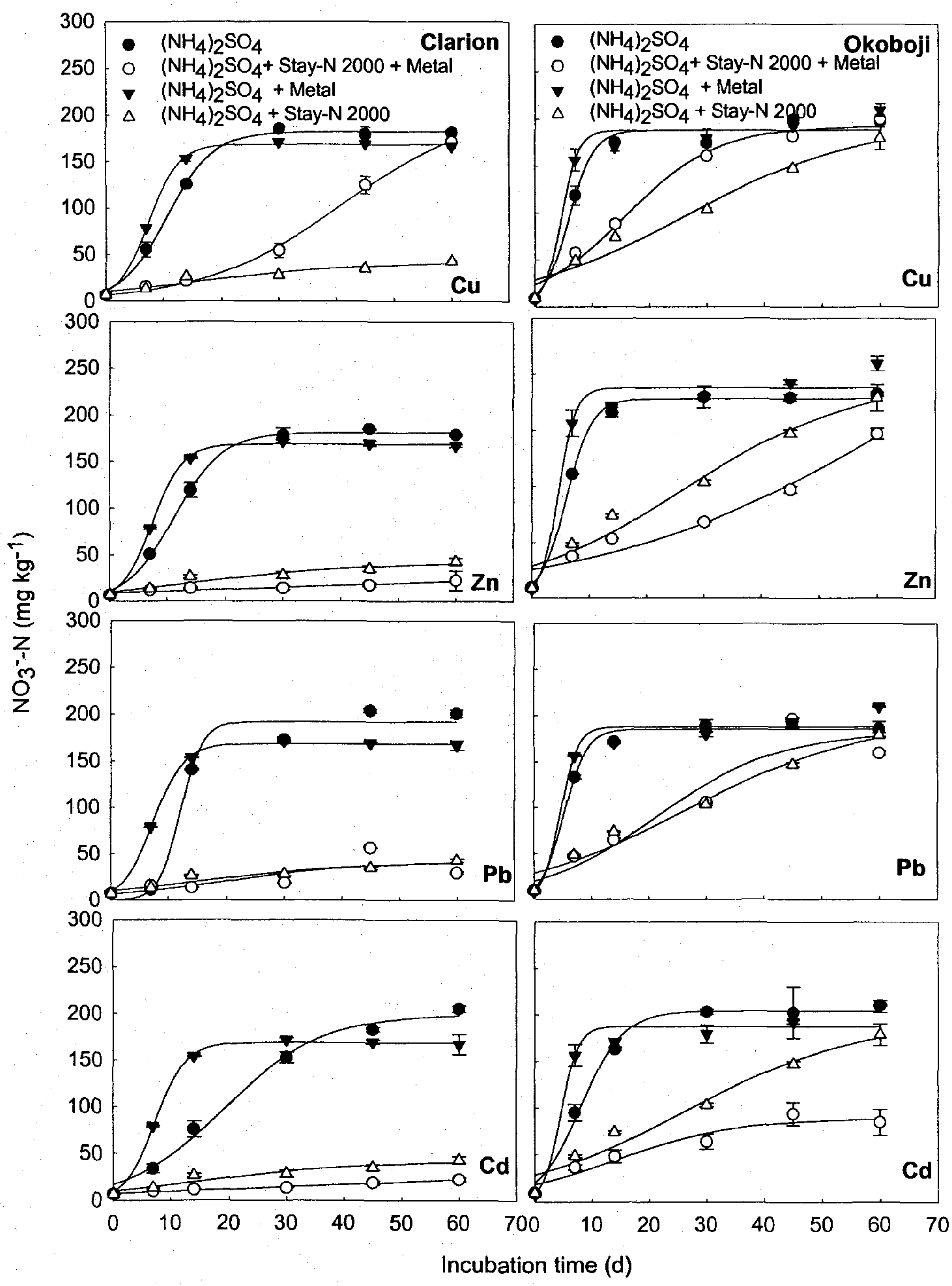

Figure 1. Nitrate-N accumulation in Iowa soils as affected by heavy metals.

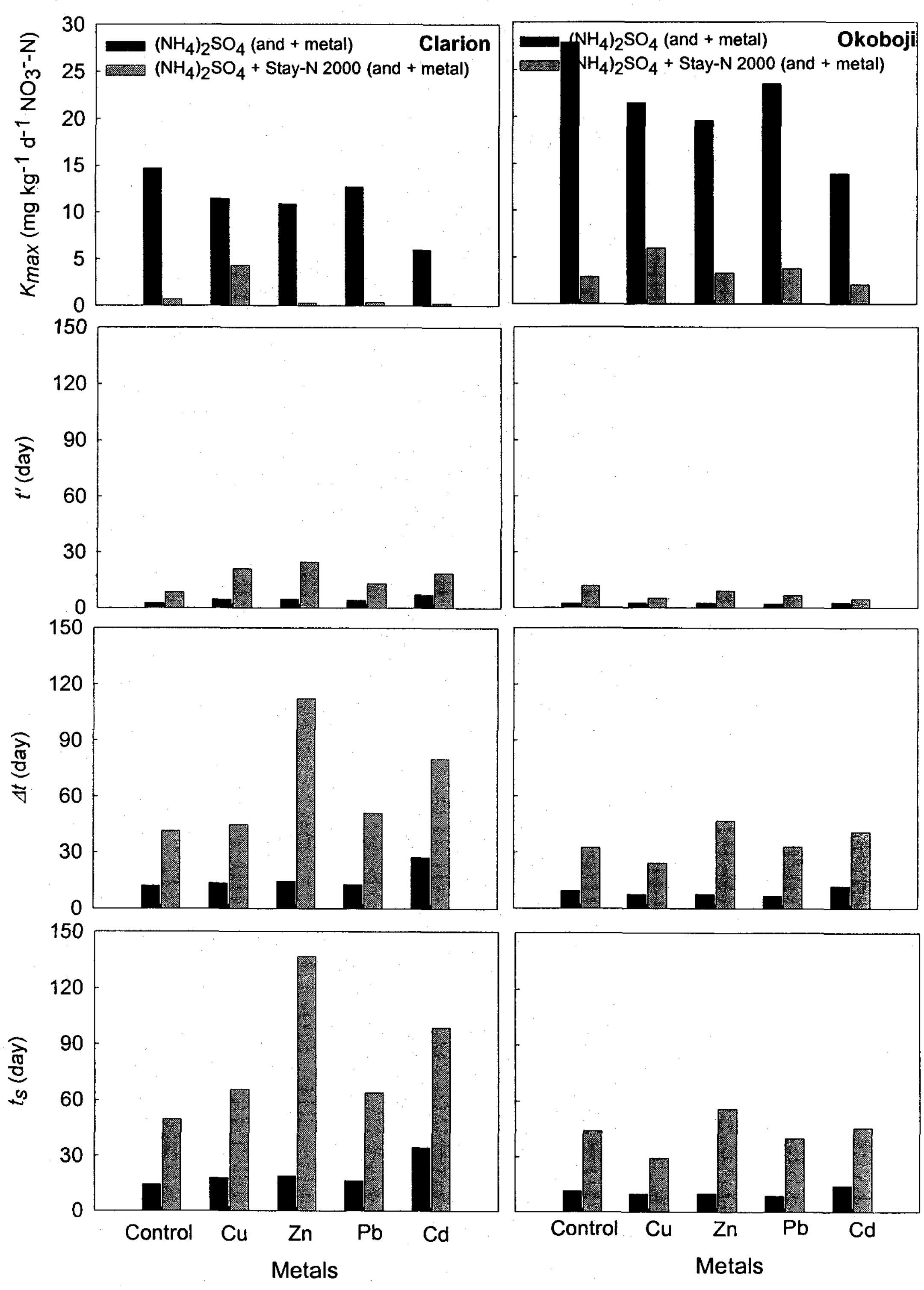

Figure 2. Calculated nitrification parameters of added $(NH_4)_2SO_4$ as affected by heavy metals.

CHAPTER 5. GENERAL CONCLUSIONS

The primary objective of this research was to characterize the kinetics of nitrification in Iowa soils treated with a new nitrification inhibitor, Stay-N 2000, or reformulated nitrapyrin, [2-chloro-6-(tricholoromethyl) pyridine], developed by Platte Chemical Co (Greeley, CO). An incubation study was performed to evaluate the performance of this compound on nitrification rates in four Iowa soils varying markedly in organic C, pH and texture. A nonlinear regression was used to determine the nitrate accumulation, followed by using first order equations to calculate the maximum nitrification rate (K_{max}), the duration of lag period (t'), the period of maximum nitrification (Δt), and the termination period of maximum nitrification (t_s).

Application of Stay-N 2000 markedly altered the course of nitrification in all Iowa soils. This study indicates that nitrification can be slowed considerably by application of Stay-N 2000 to NH_4^+-N amended soils. This inhibitor reduced the maximum nitrification rate, extended the lag period of nitrification, and subsequently extended the period of maximum nitrification as well as the termination period. Each soil exhibited different rates of nitrification. The soil type seemed to play an important role in determining the degree of Stay-N 2000 inhibition on nitrification of NH_4^+-N amended soils. The accumulation of NO_3^--N is higher in the soils with high pH and high in organic C, such as Okoboji and Canisteo than the slightly acidic soils and low in organic C, such as in Clarion and Nicollet. Organic matter is known as major sorbing component of nitrapyrin.

By comparing Stay-N 2000 and nitrapyrin, the results indicated that Stay-N 2000 was a better inhibitor in extending the lag period of nitrification of Iowa soils amended with NH_4^+-N.

There are some factors considered to be important in the efficacy of nitrification inhibitors. In our studies, we evaluated factors such as, Stay-N 2000 rates, N rates, temperature and matric potential, and heavy metals application on the rates and inhibition of nitrification in Iowa soils treated with Stay-N 2000. From the results we found that each factor has different inhibitory effect on the nitrification as indicated by different amount of nitrate accumulation, different maximum nitrification rate (K_{max}), the duration of lag period (t'), the period of maximum nitrification (Δt), and the termination period of maximum nitrification (t_s). It is recognized that all of these factors affect bioactivity and persistence of inhibitors. However, no single factor affects the overall nitrification process. They are often interactive to each other in a more complex situation that is so often the rule with natural biological systems.

APPENDIX A. Effect of Stay- N 2000 and Nitrapyrin on the accumulation of NH_4^+-N and NO_3^--N in selected Iowa soils

Soil	Incubation time											
	0 d		7 d		14 d		30 d		45 d		60 d	
	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-
	$\mu g\ g^{-1}$											
	Stay-N 2000											
Clarion												
$(NH_4)_2SO_4$	230.30	8.35	134.43	55.05	87.59	163.90	18.00	202.60	11.98	218.85	6.00	220.20
	229.70	8.95	134.70	55.35	88.02	162.75	17.98	200.40	12.00	220.65	6.00	226.30
$(NH_4)_2SO_4$ + Inh	233.74	16.05	224.20	16.95	218.30	22.61	209.80	27.10	204.30	32.59	174.98	43.82
	231.25	15.60	224.90	15.86	217.80	21.85	210.00	26.15	205.01	30.15	174.29	44.20
Okoboji												
$(NH_4)_2SO_4$	244.30	12.70	166.00	169.30	0.00	184.55	0.00	214.05	0.00	208.65	0.00	223.46
	243.10	12.40	23.60	169.05	0.00	189.90	0.00	206.75	0.00	205.65	0.00	229.60
$(NH_4)_2SO_4$ + Inh	240.90	31.88	180.00	58.00	169.00	77.65	92.21	115.90	62.04	153.80	32.00	202.36
	236.55	30.00	181.75	57.75	168.30	78.40	89.71	113.70	69.23	156.68	32.00	201.45
Nicollet												
$(NH_4)_2SO_4$	221.23	6.95	155.98	79.10	64.00	144.50	0.00	208.21	0.00	214.50	0.00	214.35
	211.73	6.90	159.50	78.40	58.00	149.50	0.00	201.35	0.00	211.13	0.00	216.71
$(NH_4)_2SO_4$ + Inh	202.34	7.05	184.35	13.80	174.20	22.70	169.50	27.15	162.34	34.10	154.00	53.50
	207.78	7.05	183.50	12.80	173.45	22.95	171.98	26.90	162.87	35.05	147.50	62.00
Canisteo												
$(NH_4)_2SO_4$	238.73	9.60	84.50	123.50	4.57	219.00	0.00	224.32	0.00	228.00	0.00	236.50
	235.67	9.85	77.00	128.50	7.45	212.00	0.00	225.71	0.00	233.00	0.00	234.50
$(NH_4)_2SO_4$ + Inh	227.42	9.25	138.50	69.50	128.00	107.45	0.36	159.72	13.35	218.50	0.56	226.71
	227.31	9.35	140.00	66.00	121.00	117.17	0.31	161.74	6.70	213.44	0.54	219.22

						Nitrapyrin						
Clarion												
$(NH_4)_2SO_4$	226.20	8.35	132.00	78.50	87.00	165.50	0.00	220.00	12.00	229.00	6.00	252.00
	234.00	8.95	153.50	70.00	86.90	157.50	0.00	224.00	12.00	234.50	6.00	254.50
$(NH_4)_2SO_4$ + Inh	232.00	16.05	213.00	22.70	210.00	23.50	188.00	37.25	189.50	55.40	170.00	60.00
	232.00	15.60	199.50	22.90	198.00	22.60	184.00	39.00	167.00	58.70	171.00	62.50
Okoboji												
$(NH_4)_2SO_4$	239.00	11.71	164.00	161.00	24.90	225.50	0.00	247.00	0.00	250.50	0.00	260.00
	243.10	12.56	168.00	155.00	25.00	227.50	0.00	253.00	0.00	255.00	0.00	253.50
$(NH_4)_2SO_4$ + Inh	240.90	41.88	180.00	83.50	161.00	121.00	131.00	174.50	77.00	212.00	0.00	249.00
	236.55	40.23	178.98	76.00	160.80	117.00	129.50	155.50	72.78	220.50	0.00	253.00
Nicollet												
$(NH_4)_2SO_4$	221.23	6.95	184.00	37.38	64.00	94.73	0.00	158.73	0.00	178.33	0.00	184.21
	211.73	6.90	182.50	41.72	58.00	89.35	0.00	161.72	0.00	189.22	0.00	206.71
$(NH_4)_2SO_4$ + Inh	202.34	7.05	192.21	13.80	174.20	22.70	169.50	27.15	162.34	34.10	154.00	53.50
	207.78	7.05	187.33	12.80	173.45	22.95	171.98	26.90	162.87	35.05	147.50	62.00
Canisteo												
$(NH_4)_2SO_4$	238.73	9.60	34.22	123.50	10.80	219.00	0.00	224.32	0.00	228.00	0.00	236.50
	235.67	9.85	37.41	128.50	16.76	212.00	0.00	225.71	0.00	233.00	0.00	234.50
$(NH_4)_2SO_4$ + Inh	227.42	9.25	138.50	69.50	128.00	107.45	0.36	159.72	13.35	218.50	0.00	226.71
	227.31	9.35	140.00	66.00	121.00	117.17	0.31	161.74	6.70	213.44	0.00	219.22

APPENDIX B. Effect of Stay- N 2000 rates on the accumulation of NH_4^+-N and NO_3^--N in selected Iowa soils

	Incubation time											
	0 d		7 d		14 d		30 d		45 d		60 d	
Soil	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-
	$\mu g\ g^{-1}$											
	4 $\mu g\ g^{-1}$ a.i Stay-N 2000											
Clarion												
$(NH_4)_2SO_4$	220.00	8.35	140.00	55.05	124.30	163.90	100.00	202.60	24.30	218.85	0.00	220.20
	220.00	8.95	138.00	55.35	122.98	162.75	102.00	200.40	24.00	220.65	0.00	226.30
$(NH_4)_2SO_4$ + Inh	224.00	16.05	218.00	16.95	210.00	22.61	196.00	27.10	188.00	32.59	144.00	43.82
	224.00	15.60	218.00	15.86	218.30	21.85	194.00	26.15	182.00	30.15	145.00	44.20
Okoboji												
$(NH_4)_2SO_4$	234.00	12.70	43.00	169.30	0.00	184.55	0.00	214.05	0.00	208.65	0.00	223.46
	228.00	12.40	43.00	169.05	0.00	189.90	0.00	206.75	0.00	205.65	0.00	229.60
$(NH_4)_2SO_4$ + Inh	238.00	31.88	196.00	58.00	161.00	77.65	130.25	115.90	77.00	153.80	7.34	202.36
	237.98	30.00	196.00	57.75	161.00	78.40	132.00	113.70	73.00	156.68	7.36	201.45
Nicollet												
$(NH_4)_2SO_4$	220.43	7.05	165.34	73.21	64.32	147.85	32.21	188.72	0.00	198.56	0.00	207.53
	221.57	7.09	159.50	78.63	68.71	148.54	30.78	181.27	0.00	201.50	0.00	202.11
$(NH_4)_2SO_4$ + Inh	212.34	7.05	183.21	13.80	170.32	22.70	159.63	27.15	152.49	34.10	144.22	53.50
	217.58	7.05	183.55	12.80	173.25	22.95	155.34	26.90	152.71	35.05	147.50	62.00
Canisteo												
$(NH_4)_2SO_4$	235.62	9.60	74.53	123.50	7.98	219.00	0.00	224.32	0.00	228.00	0.00	236.50
	238.67	9.85	77.00	128.50	7.26	212.00	0.00	225.71	0.00	233.00	0.00	234.50
$(NH_4)_2SO_4$ + Inh	227.42	9.25	133.29	69.50	125.78	107.45	111.36	159.72	93.35	218.50	87.56	226.71
	221.36	9.35	141.20	66.00	127.65	117.17	110.31	161.74	96.70	213.44	89.54	219.22

8 mg g^{-1} a.i Stay-N 2000

Clarion												
$(NH_4)_2SO_4$	220.00	8.35	140.00	79.10	124.30	160.50	100.00	165.50	24.30	205.00	0.00	208.00
	220.00	8.95	138.00	78.40	122.98	160.50	102.00	162.50	24.00	207.50	0.00	214.00
$(NH_4)_2SO_4$ + Inh	224.00	16.05	211.00	13.80	201.00	10.45	192.00	8.75	188.00	14.85	166.00	24.85
	224.00	15.60	211.00	12.80	214.00	11.60	190.00	8.35	184.00	15.25	166.00	31.50
Okoboji												
$(NH_4)_2SO_4$	234.00	11.71	43.00	145.00	0.00	198.50	0.00	205.50	0.00	208.00	0.00	212.00
	228.00	12.56	43.00	138.50	0.00	200.50	0.00	206.00	0.00	208.50	0.00	213.00
$(NH_4)_2SO_4$ + Inh	238.00	41.88	144.00	36.70	140.00	42.95	130.25	59.00	105.00	87.00	105.00	122.00
	237.98	40.23	144.00	36.25	140.00	45.95	132.00	60.50	105.00	85.50	105.00	121.00
Nicollet												
$(NH_4)_2SO_4$	221.23	10.85	184.00	56.32	64.00	84.73	12.10	158.73	0.00	171.53	0.00	179.83
	211.73	10.90	182.50	51.42	58.00	89.27	11.57	155.27	0.00	179.52	0.00	184.23
$(NH_4)_2SO_4$ + Inh	202.34	7.55	192.21	13.89	174.20	22.70	160.31	28.15	156.25	34.23	144.32	35.25
	207.78	7.32	187.33	14.20	173.45	22.95	151.25	26.32	152.27	35.11	147.31	36.02
Canisteo												
$(NH_4)_2SO_4$	238.73	10.60	134.22	133.50	12.83	199.81	0.00	217.39	0.00	223.69	0.00	229.65
	235.67	10.85	137.55	138.54	16.34	192.63	0.00	222.94	0.00	225.74	0.00	227.54
$(NH_4)_2SO_4$ + Inh	227.42	9.25	168.72	39.50	158.91	40.45	133.36	49.72	14.35	58.50	0.00	66.71
	227.31	9.35	170.31	36.00	161.73	41.17	131.25	51.74	16.76	53.44	0.00	69.87

12 mg g^{-1} a.i Stay-N 2000

Clarion												
$(NH_4)_2SO_4$	220.00	8.35	140.00	83.00	124.30	203.00	100.00	203.00	24.30	207.50	0.00	213.00
	220.00	8.95	138.00	80.00	122.98	199.00	102.00	202.50	24.00	203.50	0.00	209.00
$(NH_4)_2SO_4$ + Inh	224.00	16.05	210.00	9.20	207.00	13.45	193.00	12.35	185.36	10.20	165.00	11.30
	224.00	15.60	210.00	9.40	207.00	10.45	192.00	13.10	189.32	7.50	165.00	11.50
Okoboji												
$(NH_4)_2SO_4$	234.00	11.71	43.00	139.00	0.00	194.50	0.00	201.50	0.00	211.00	0.00	215.00
	228.00	12.56	43.00	134.00	0.00	195.50	0.00	202.00	0.00	206.50	0.00	215.00
$(NH_4)_2SO_4$ + Inh	238.00	11.88	187.00	23.90	155.00	25.60	133.00	31.40	120.00	39.55	117.00	54.50
	237.98	10.23	187.00	24.65	157.00	26.90	137.00	31.90	123.98	41.35	117.00	55.00
Nicollet												
$(NH_4)_2SO_4$	220.31	8.95	144.32	57.68	64.00	94.73	0.00	158.63	0.00	178.33	0.00	184.21
	211.83	8.90	142.59	51.43	58.00	89.35	0.00	161.17	0.00	189.22	0.00	206.71
$(NH_4)_2SO_4$ + Inh	197.34	9.05	172.69	11.23	174.20	18.70	169.50	20.15	162.34	14.12	135.00	11.90
	200.71	9.51	177.58	10.45	173.45	16.45	171.98	22.43	162.87	15.32	137.50	12.30
Canisteo												
$(NH_4)_2SO_4$	228.36	9.42	44.58	103.31	10.80	189.65	0.00	204.61	0.00	208.00	0.00	210.50
	225.54	9.75	47.58	108.45	16.76	192.33	0.00	205.36	0.00	213.00	0.00	214.50
$(NH_4)_2SO_4$ + Inh	217.79	9.32	133.64	29.50	128.00	37.45	132.36	38.72	13.35	39.50	0.00	42.80
	207.53	9.65	140.00	26.42	121.00	37.17	133.41	41.74	6.70	41.44	0.00	43.10

APPENDIX C. Effect of N rates on the accumulation of NH_4^+-N and NO_3^--N in selected Iowa soils treated with Stay-N 2000

	Incubation time											
	0 d		7 d		14 d		30 d		45 d		60 d	
Soil	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-
	$\mu g\ g^{-1}$											
	0.5 mg-N											
Clarion												
$(NH_4)_2SO_4$	68.50	16.08	54.85	52.45	0.00	67.55	0.00	77.55	0.00	78.80	0.00	81.83
	66.20	16.40	56.40	54.00	0.00	68.40	0.00	76.40	0.00	80.75	0.00	83.75
$(NH_4)_2SO_4$ + Inh	67.60	10.98	58.30	15.93	57.85	18.80	59.25	24.40	56.10	23.60	42.50	31.07
	66.05	10.25	59.20	15.73	57.80	19.84	58.10	23.05	54.70	24.60	43.40	32.36
Okoboji												
$(NH_4)_2SO_4$	62.60	38.98	0.00	62.15	0.00	73.50	0.00	78.15	0.00	74.05	0.00	77.45
	61.25	39.70	0.00	59.30	0.00	73.60	0.00	76.80	0.00	78.30	0.00	75.90
$(NH_4)_2SO_4$ + Inh	61.30	21.99	17.29	55.08	7.65	56.10	0.00	59.80	0.00	75.55	0.00	74.95
	60.55	22.61	17.66	53.20	7.07	56.35	0.00	62.15	0.00	74.95	0.00	81.45
Nicollet												
$(NH_4)_2SO_4$	58.25	17.08	44.75	50.45	0.00	57.52	0.00	77.55	0.00	80.80	0.00	81.23
	56.32	16.40	52.82	55.00	0.00	58.34	0.00	79.40	0.00	80.75	0.00	79.75
$(NH_4)_2SO_4$ + Inh	57.60	11.68	44.60	15.68	30.90	19.68	29.60	24.68	27.70	30.08	24.70	34.17
	56.05	9.27	43.05	13.27	29.35	17.27	28.05	22.27	26.15	28.67	23.15	32.01
Canisteo												
$(NH_4)_2SO_4$	62.67	34.98	0.00	52.84	0.00	63.64	0.00	73.15	0.00	74.76	0.00	77.54
	64.37	37.47	0.00	57.31	0.00	66.70	0.00	73.49	0.00	75.87	0.00	79.91
$(NH_4)_2SO_4$ + Inh	51.73	21.99	17.29	35.99	7.65	49.82	0.00	63.82	0.00	70.26	0.00	74.95
	50.59	22.61	17.66	36.61	7.07	51.76	0.00	58.45	0.00	79.81	0.00	81.45

						1 mg-N						
Clarion												
$(NH_4)_2SO_4$	148.40	7.70	57.25	52.56	0.00	88.05	0.00	101.35	0.00	108.65	0.00	112.34
	146.70	6.55	59.21	52.80	0.00	87.95	0.00	104.50	0.00	106.70	0.00	112.50
$(NH_4)_2SO_4$ + Inh	139.65	10.05	108.05	17.34	95.69	18.00	87.54	21.31	86.02	27.64	92.03	33.44
	137.50	9.95	109.40	16.05	95.29	18.13	90.06	21.75	84.57	27.64	91.17	32.95
Okoboji												
$(NH_4)_2SO_4$	143.85	6.74	0.00	100.79	0.00	104.89	0.00	111.05	0.00	112.05	0.00	114.35
	141.95	6.42	0.00	101.49	0.00	107.63	0.00	110.50	0.00	113.50	0.00	114.80
$(NH_4)_2SO_4$ + Inh	135.15	22.80	43.48	63.35	31.50	77.45	12.02	81.71	0.00	91.26	0.00	109.92
	135.05	22.50	41.08	61.78	31.26	78.25	13.50	76.55	0.00	85.20	0.00	109.36
Nicollet												
$(NH_4)_2SO_4$	148.74	7.89	75.23	52.56	33.87	88.05	0.00	101.35	0.00	108.65	0.00	110.56
	142.71	7.65	69.99	52.80	38.29	87.95	0.00	104.50	0.00	106.70	0.00	112.78
$(NH_4)_2SO_4$ + Inh	149.65	10.89	136.65	17.53	122.95	19.21	121.05	23.31	119.15	28.59	106.25	32.44
	147.56	11.75	134.56	18.23	120.86	20.43	118.96	14.57	117.06	27.54	104.16	30.21
Canisteo												
$(NH_4)_2SO_4$	153.85	8.74	0.00	70.89	0.00	98.89	0.00	110.73	0.00	112.54	0.00	113.96
	151.95	10.42	0.00	81.72	0.00	87.74	0.00	110.65	0.00	111.78	0.00	112.89
$(NH_4)_2SO_4$ + Inh	139.15	25.83	33.48	54.25	30.69	72.54	5.02	79.71	0.00	89.34	0.00	94.72
	133.25	23.74	31.89	51.89	29.26	70.71	6.50	75.64	0.00	85.76	0.00	90.29

2 mg-N

Clarion												
$(NH_4)_2SO_4$	226.20	8.35	173.43	55.05	47.59	163.90	0.00	202.60	0.00	218.85	0.00	220.20
	227.25	8.95	176.55	55.35	49.42	162.75	0.00	200.40	0.00	220.65	0.00	226.30
$(NH_4)_2SO_4$ + Inh	226.20	16.05	204.20	16.95	131.99	22.61	125.61	27.10	109.90	32.59	104.65	43.82
	227.25	15.60	207.90	15.86	133.26	21.85	124.74	26.15	108.92	30.15	105.83	44.20
Okoboji												
$(NH_4)_2SO_4$	244.30	12.70	22.62	169.30	0.00	184.55	0.00	214.05	0.00	208.65	0.00	223.46
	243.10	12.40	23.60	169.05	0.00	189.90	0.00	206.75	0.00	205.65	0.00	229.60
$(NH_4)_2SO_4$ + Inh	240.90	31.88	162.60	58.00	101.93	77.65	92.21	115.90	62.04	153.80	13.16	202.36
	236.55	30.00	158.93	57.75	102.27	78.40	89.71	113.70	59.46	156.68	14.95	201.45
Nicollet												
$(NH_4)_2SO_4$	217.28	10.78	183.20	49.23	37.48	157.02	0.00	199.34	0.00	215.54	0.00	216.17
	217.25	10.67	180.37	52.57	39.21	153.37	0.00	194.37	0.00	210.75	0.00	212.37
$(NH_4)_2SO_4$ + Inh	216.72	12.37	212.56	17.85	154.23	22.61	134.76	31.42	100.45	38.84	104.65	40.72
	217.83	11.63	210.93	17.24	149.73	21.85	137.51	36.78	102.43	38.02	105.83	41.73
Canisteo												
$(NH_4)_2SO_4$	240.38	42.70	122.71	154.82	0.00	178.54	0.00	201.55	0.00	208.40	0.00	213.71
	243.19	42.56	127.76	159.70	0.00	179.03	0.00	207.31	0.00	205.71	0.00	219.52
$(NH_4)_2SO_4$ + Inh	239.94	34.88	162.60	51.40	122.76	65.71	94.20	125.37	54.21	143.22	19.73	153.21
	236.77	34.17	158.93	57.78	120.38	68.94	91.70	123.89	59.72	146.41	18.71	157.76

APPENDIX D. Effect of temperatures and soil matric potentials on the accumulation of NH_4^+-N and NO_3^--N in selected Iowa soils treated with Stay-N 2000

	Incubation time											
	0 d		7 d		14 d		30 d		45 d		60 d	
Soil	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-
	---μg g^{-1}---											
	10°C, -1 kPa											
Clarion												
$(NH_4)_2SO_4$	187.43	6.75	180.37	52.45	144.71	107.30	123.45	147.55	64.33	178.80	35.67	181.83
	183.27	6.95	175.33	54.00	142.98	108.67	121.43	146.60	69.71	180.75	34.73	183.30
$(NH_4)_2SO_4$ + Inh	173.23	7.84	169.37	15.93	157.85	18.80	148.76	24.40	126.71	28.60	42.50	31.07
	176.21	7.98	169.33	15.73	157.80	19.84	143.78	23.05	124.75	29.77	43.40	32.36
Okoboji												
$(NH_4)_2SO_4$	190.32	11.55	132.44	62.15	64.78	143.50	32.55	178.17	0.00	184.05	0.00	187.45
	192.43	12.52	133.46	59.30	69.70	143.67	33.71	178.60	0.00	188.34	0.00	190.90
$(NH_4)_2SO_4$ + Inh	192.32	10.45	154.66	55.08	133.70	86.72	107.44	109.80	87.35	165.55	64.73	174.95
	193.24	10.27	157.89	53.20	133.23	86.24	108.34	102.43	86.44	164.07	63.89	181.22
Nicollet												
$(NH_4)_2SO_4$	190.00	6.75	181.00	18.30	169.50	20.75	114.00	72.50	55.50	124.50	28.95	133.50
	193.22	6.95	182.50	18.62	163.00	22.30	125.00	76.75	71.50	129.00	21.15	139.00
$(NH_4)_2SO_4$ + Inh	199.78	7.30	189.50	9.30	171.00	13.55	156.50	18.09	148.33	27.25	121.00	34.92
	201.22	7.30	189.50	9.50	172.00	14.80	158.00	17.41	145.99	29.20	125.77	34.31
Canisteo												
$(NH_4)_2SO_4$	197.54	9.55	147.50	12.90	88.00	60.00	0.00	122.50	0.00	128.00	0.00	135.50
	195.33	10.85	147.00	13.80	82.00	66.50	0.00	126.00	0.00	130.00	0.00	140.00
$(NH_4)_2SO_4$ + Inh	201.77	9.45	165.50	12.42	158.50	19.95	157.00	30.10	145.33	42.40	135.00	43.52
	209.34	9.45	175.00	12.46	166.00	17.50	160.00	32.80	144.32	47.35	137.22	47.50

20°C, -1 kPa

Clarion												
$(NH_4)_2SO_4$	187.43	6.75	170.05	55.18	126.94	108.23	110.24	164.16	53.87	184.43	20.70	182.21
	183.27	6.95	165.01	56.73	125.21	109.78	108.22	165.71	56.25	175.98	19.76	183.76
$(NH_4)_2SO_4$ + Inh	173.23	7.84	159.05	18.66	140.08	24.66	135.55	24.78	113.25	33.28	27.53	35.35
	176.21	7.98	159.01	18.46	141.23	21.37	130.57	24.66	111.29	38.08	29.73	35.15
Okoboji												
$(NH_4)_2SO_4$	190.32	11.55	91.68	42.38	61.46	133.72	41.07	170.14	0.00	189.05	0.00	189.75
	192.43	12.52	92.70	40.31	62.48	136.54	42.09	172.42	0.00	192.32	0.00	190.39
$(NH_4)_2SO_4$ + Inh	192.32	10.45	163.90	35.08	124.33	56.72	99.58	99.80	67.33	155.24	44.73	179.54
	193.24	10.27	177.91	33.21	116.98	59.63	92.23	97.54	66.31	154.32	43.28	180.35
Nicollet												
$(NH_4)_2SO_4$	171.23	6.75	173.00	9.55	118.50	48.80	22.10	126.00	3.22	163.00	1.12	172.00
	173.25	6.95	179.00	8.55	118.00	48.45	25.30	136.00	3.45	158.50	0.89	167.00
$(NH_4)_2SO_4$ + Inh	169.33	3.30	159.50	6.00	141.00	15.75	131.50	24.19	117.36	37.05	85.50	40.64
	173.65	3.30	158.00	5.09	145.00	16.66	137.50	23.85	120.35	36.30	81.17	39.80
Canisteo												
$(NH_4)_2SO_4$	180.33	5.55	50.50	71.50	84.00	84.00	0.00	88.50	0.00	97.00	0.00	100.50
	187.23	6.85	61.50	70.50	90.00	85.00	0.00	92.00	0.00	96.50	0.00	104.00
$(NH_4)_2SO_4$ + Inh	181.45	5.45	168.50	7.52	164.50	20.06	58.50	46.00	15.20	80.50	0.00	89.50
	185.23	5.45	153.50	7.50	161.00	22.61	86.00	52.20	19.45	80.00	0.00	85.50

30°C, -1 kPa

Clarion												
$(NH_4)_2SO_4$	187.43	6.75	170.05	55.18	126.94	108.23	110.24	164.16	53.87	184.43	20.70	182.21
	183.27	6.95	165.01	56.73	125.21	109.78	108.22	165.71	56.25	175.98	19.76	183.76
$(NH_4)_2SO_4$ + Inh	173.23	7.84	159.05	18.66	140.08	24.66	135.55	24.78	113.25	33.28	27.53	35.35
	176.21	7.98	159.01	18.46	141.23	21.37	130.57	24.66	111.29	38.08	29.73	35.15
Okoboji												
$(NH_4)_2SO_4$	190.32	11.55	91.68	42.38	61.46	133.72	41.07	170.14	0.00	189.05	0.00	189.75
	192.43	12.52	92.70	40.31	62.48	136.54	42.09	172.42	0.00	192.32	0.00	190.39
$(NH_4)_2SO_4$ + Inh	192.32	10.45	163.90	35.08	124.33	56.72	99.58	99.80	67.33	155.24	44.73	179.54
	193.24	10.27	177.91	33.21	116.98	59.63	92.23	97.54	66.31	154.32	43.28	180.35
Nicollet												
$(NH_4)_2SO_4$	161.23	6.75	131.00	57.33	119.00	83.80	74.95	114.80	32.58	154.50	14.37	159.23
	173.25	6.95	133.25	59.88	116.00	80.00	77.24	115.23	32.56	161.72	15.96	157.71
$(NH_4)_2SO_4$ + Inh	169.33	3.30	157.64	6.64	144.36	21.74	125.50	42.71	116.00	44.60	63.10	52.31
	173.65	3.30	152.00	8.75	142.30	21.66	130.31	42.73	112.32	44.30	66.90	48.72
Canisteo												
$(NH_4)_2SO_4$	180.33	5.55	48.55	33.00	7.00	77.15	0.00	104.63	0.00	169.88	0.00	177.55
	187.23	6.85	44.00	32.11	6.30	74.80	0.00	101.32	0.00	167.34	0.00	176.23
$(NH_4)_2SO_4$ + Inh	181.45	5.45	172.00	11.70	82.50	21.50	33.18	35.70	21.11	43.71	9.54	45.22
	185.23	5.45	165.00	12.31	79.00	22.05	31.28	37.55	19.98	42.34	10.76	47.32

10°C, -10 kPa

Clarion												
$(NH_4)_2SO_4$	187.43	7.54	167.16	48.89	152.81	92.45	140.81	127.54	64.32	178.80	45.67	184.00
	183.27	8.21	162.12	50.44	147.77	94.00	135.77	126.32	66.71	180.75	44.32	183.00
$(NH_4)_2SO_4$ + Inh	173.23	7.82	156.16	12.37	142.63	21.93	129.81	24.40	116.32	27.00	51.00	33.07
	176.21	7.54	156.12	12.17	141.77	18.61	129.77	23.05	114.71	29.66	54.36	33.24
Okoboji												
$(NH_4)_2SO_4$	190.32	11.23	119.23	58.59	105.88	132.15	52.88	184.32	0.00	188.00	0.00	190.00
	192.43	11.52	120.25	55.74	105.90	129.30	52.90	188.32	0.00	189.31	0.00	191.23
$(NH_4)_2SO_4$ + Inh	192.32	10.45	141.45	31.52	133.10	45.08	74.10	113.54	57.34	165.55	24.73	174.95
	193.24	10.27	144.68	29.64	130.33	43.20	77.33	117.56	56.33	164.07	23.98	181.22
Nicollet												
$(NH_4)_2SO_4$	171.72	6.75	176.69	28.30	154.62	34.90	124.92	37.01	112.45	47.74	107.83	49.29
	174.25	6.95	172.74	27.90	155.58	32.75	124.37	37.39	114.80	47.36	101.40	50.54
$(NH_4)_2SO_4$ + Inh	179.85	3.30	182.15	20.63	173.20	24.95	151.23	23.86	145.25	25.95	129.83	24.15
	173.65	3.30	198.75	18.85	175.92	22.54	153.66	23.68	142.80	24.60	126.17	23.12
Canisteo												
$(NH_4)_2SO_4$	180.33	5.55	119.00	74.90	33.88	153.45	0.00	179.90	0.00	182.00	0.00	187.50
	187.23	6.85	112.50	74.54	34.91	151.01	0.00	179.12	0.00	182.58	0.00	191.10
$(NH_4)_2SO_4$ + Inh	183.25	5.45	170.10	23.07	164.20	27.28	141.48	48.74	134.65	34.61	117.31	35.80
	185.23	5.45	171.50	23.37	161.40	29.00	140.14	46.81	139.79	35.65	114.84	37.60

						20°C, -10 kPa						
Clarion												
$(NH_4)_2SO_4$	187.43	5.03	121.36	63.80	135.19	163.90	15.21	144.92	0.00	161.48	0.00	177.30
	183.27	5.36	121.42	65.35	139.42	157.10	16.77	150.42	0.00	161.72	0.00	174.11
$(NH_4)_2SO_4$ + Inh	173.23	12.40	140.68	17.18	131.99	22.61	125.61	28.20	109.90	30.10	92.03	33.21
	176.21	11.43	139.13	15.86	133.26	23.65	124.74	27.43	108.92	36.52	91.17	35.32
Okoboji												
$(NH_4)_2SO_4$	190.32	7.40	22.62	59.30	0.00	104.55	0.00	132.08	0.00	158.27	0.00	190.00
	192.43	7.97	23.60	59.03	0.00	105.30	0.00	143.82	0.00	160.67	0.00	190.75
$(NH_4)_2SO_4$ + Inh	192.32	11.88	121.08	28.00	101.93	57.65	92.21	74.08	62.04	106.36	28.76	148.70
	193.24	17.23	117.46	31.00	102.27	58.40	89.71	67.08	59.46	107.58	37.65	146.76
Nicollet												
$(NH_4)_2SO_4$	161.23	6.75	166.50	97.01	103.90	141.23	71.65	193.30	35.12	211.90	13.23	211.94
	173.25	6.95	171.10	97.05	97.15	141.60	78.25	191.20	36.41	206.96	13.61	194.71
$(NH_4)_2SO_4$ + Inh	169.33	3.30	203.65	26.14	195.30	30.25	185.35	34.93	165.45	42.54	145.75	53.07
	173.65	3.30	213.58	26.54	195.20	30.03	185.32	32.88	166.60	40.71	142.90	51.76
Canisteo												
$(NH_4)_2SO_4$	180.33	5.55	0.00	149.05	0.00	177.70	0.00	193.20	0.00	200.80	0.00	207.80
	187.23	6.85	0.00	148.95	0.00	178.40	0.00	201.10	0.00	207.19	0.00	205.80
$(NH_4)_2SO_4$ + Inh	181.45	5.45	204.30	38.10	200.25	40.85	181.45	46.70	149.65	61.50	124.65	76.20
	185.23	5.45	204.20	38.75	199.65	40.75	177.45	46.81	153.60	61.44	134.90	76.57

30°C, -10 kPa

Clarion												
$(NH_4)_2SO_4$	187.43	6.75	54.85	52.45	0.00	67.55	0.00	77.55	0.00	78.80	0.00	81.83
	183.27	6.95	56.40	54.00	0.00	68.40	0.00	76.40	0.00	80.75	0.00	83.75
$(NH_4)_2SO_4$ + Inh	173.23	7.84	58.30	15.93	57.85	18.80	59.25	24.40	56.10	23.60	42.50	31.07
	176.21	7.98	59.20	15.73	57.80	19.84	58.10	23.05	54.70	24.60	43.40	32.36
Okoboji												
$(NH_4)_2SO_4$	190.32	11.55	0.00	62.15	0.00	73.50	0.00	78.15	0.00	74.05	0.00	77.45
	192.43	12.52	0.00	59.30	0.00	73.60	0.00	76.80	0.00	78.30	0.00	75.90
$(NH_4)_2SO_4$ + Inh	192.32	10.45	17.29	55.08	7.65	56.10	0.00	59.80	0.00	75.55	0.00	74.95
	193.24	10.27	17.66	53.20	7.07	56.35	0.00	62.15	0.00	74.95	0.00	81.45
Nicollet												
$(NH_4)_2SO_4$	161.23	6.75	138.00	95.43	65.40	150.00	24.42	188.36	0.00	234.50	0.00	232.84
	173.25	6.95	144.15	95.44	59.80	151.35	25.33	187.50	0.00	235.44	0.00	243.80
$(NH_4)_2SO_4$ + Inh	169.33	3.30	218.00	30.65	215.70	37.90	203.45	57.58	181.55	88.50	173.85	101.65
	173.65	3.30	219.95	30.82	216.75	38.68	197.50	57.17	188.10	88.45	161.75	102.75
Canisteo												
$(NH_4)_2SO_4$	180.33	5.55	0.00	154.10	0.00	173.92	0.00	189.70	0.00	193.88	0.00	214.71
	187.23	6.85	0.00	151.16	0.00	172.55	0.00	184.75	0.00	201.15	0.00	191.99
$(NH_4)_2SO_4$ + Inh	181.45	5.45	171.15	36.95	143.25	53.00	53.07	128.00	0.00	180.15	0.00	188.75
	185.23	5.45	176.40	39.62	144.75	52.80	60.30	125.50	0.00	180.38	0.00	192.19

10°C, -60 kPa

Clarion												
$(NH_4)_2SO_4$	187.00	7.01	171.25	21.30	160.02	57.89	149.36	133.00	127.33	157.00	85.00	179.32
	183.00	6.95	176.96	21.65	165.73	59.23	155.07	138.00	128.54	159.00	84.74	185.00
$(NH_4)_2SO_4$ + Inh	199.80	7.84	183.46	9.60	172.23	14.70	169.57	27.92	155.50	30.45	132.00	33.40
	198.24	7.98	186.46	9.65	175.23	15.40	164.57	27.45	154.32	28.36	133.00	34.80
Okoboji												
$(NH_4)_2SO_4$	190.32	11.55	154.23	54.00	143.00	93.35	52.34	157.44	0.00	177.00	0.00	189.36
	192.43	12.52	159.64	53.65	148.41	88.32	57.68	160.36	0.00	179.00	0.00	190.00
$(NH_4)_2SO_4$ + Inh	192.32	10.45	182.69	14.10	171.46	23.40	144.80	30.05	112.00	38.90	55.50	45.00
	193.24	10.27	189.46	14.50	178.23	22.35	138.24	31.04	115.00	41.75	54.20	49.25
Nicollet												
$(NH_4)_2SO_4$	201.42	9.75	191.50	18.30	180.00	27.65	147.00	56.35	127.50	67.50	115.00	67.50
	203.88	9.95	186.50	18.65	179.50	29.05	145.50	56.15	127.00	65.50	105.00	69.50
$(NH_4)_2SO_4$ + Inh	200.55	5.40	193.00	9.60	174.00	14.70	202.50	25.90	203.50	28.45	205.00	33.40
	200.65	5.23	196.00	9.65	198.50	15.40	203.50	27.45	203.00	27.65	213.50	34.80
Canisteo												
$(NH_4)_2SO_4$	200.30	4.55	136.50	64.00	76.50	113.00	12.15	177.00	0.00	187.50	0.00	191.00
	207.30	6.85	139.00	63.50	70.00	118.00	7.90	168.50	0.00	187.00	0.00	185.00
$(NH_4)_2SO_4$ + Inh	201.52	7.53	132.50	14.10	161.00	19.00	156.00	32.05	152.00	40.90	151.50	45.00
	202.41	7.45	139.00	13.50	155.50	19.50	160.00	32.80	155.00	43.75	158.50	49.25

20°C, -60 kPa

Clarion												
$(NH_4)_2SO_4$	187.00	7.01	150.59	34.23	126.93	78.80	87.34	137.55	54.33	169.44	38.23	176.25
	183.00	6.95	152.62	36.92	128.89	73.30	86.31	134.40	58.23	168.33	41.35	188.95
$(NH_4)_2SO_4$ + Inh	199.80	7.84	17.05	16.66	38.35	45.77	75.40	66.30	109.10	71.71	133.35	76.25
	198.24	7.98	19.10	17.58	37.45	47.18	81.40	66.80	103.05	77.7	135.70	75.05
Okoboji												
$(NH_4)_2SO_4$	190.32	11.55	132.21	62.15	90.00	134.96	45.33	178.96	11.23	184.05	0.00	189.32
	192.43	12.52	134.25	59.30	93.86	133.65	42.31	176.21	13.24	188.32	0.00	190.00
$(NH_4)_2SO_4$ + Inh	192.32	10.45	177.29	55.08	154.87	56.10	115.62	129.90	65.34	165.30	33.30	177.89
	193.24	10.27	178.66	53.20	157.85	56.35	114.87	130.31	66.71	174.31	36.45	161.23
Nicollet												
$(NH_4)_2SO_4$	201.42	9.75	172.00	6.95	161.50	48.10	91.00	119.00	31.00	185.00	14.80	201.00
	203.88	9.95	171.50	6.90	159.50	49.20	93.50	117.50	62.00	154.00	20.10	197.00
$(NH_4)_2SO_4$ + Inh	200.55	5.40	172.50	7.05	194.00	17.30	192.50	24.70	183.50	38.95	176.00	54.50
	200.65	5.23	171.00	7.05	195.00	17.50	192.00	24.25	184.00	40.30	173.50	55.00
Canisteo												
$(NH_4)_2SO_4$	200.30	4.55	148.00	9.60	48.30	140.50	0.00	192.50	0.00	202.50	0.00	204.00
	207.30	6.85	145.50	9.85	52.00	135.50	0.00	190.00	0.00	199.00	0.00	200.00
$(NH_4)_2SO_4$ + Inh	201.52	7.53	147.00	9.25	155.50	34.90	143.50	46.70	125.50	68.00	85.00	106.50
	202.41	7.45	147.00	9.35	156.00	35.15	142.50	48.55	127.00	67.50	93.00	100.50

30°C, -60 kPa

Clarion												
$(NH_4)_2SO_4$	187.00	7.01	160.71	26.95	110.25	48.10	79.71	129.00	19.71	155.00	13.51	179.00
	183.00	6.95	160.23	26.90	108.21	49.20	82.21	127.00	20.73	154.00	18.54	177.00
$(NH_4)_2SO_4$ + Inh	199.80	7.84	161.21	7.05	142.21	17.30	131.23	24.70	172.21	38.95	164.71	44.50
	198.24	7.98	162.37	7.05	143.56	17.50	130.56	24.25	172.71	40.30	162.21	45.00
Okoboji												
$(NH_4)_2SO_4$	190.32	11.55	136.71	59.60	67.02	140.50	21.33	188.50	0.00	189.22	0.00	190.00
	192.43	12.52	134.21	59.85	69.31	135.50	20.48	180.23	0.00	182.73	0.00	188.00
$(NH_4)_2SO_4$ + Inh	192.32	10.45	155.71	39.25	114.21	64.91	82.21	136.75	54.21	188.00	13.72	190.00
	193.24	10.27	155.96	39.35	114.36	65.15	81.73	138.45	55.78	187.00	11.89	189.00
Nicollet												
$(NH_4)_2SO_4$	201.42	9.75	143.50	61.50	89.00	139.00	38.75	188.50	23.50	209.00	6.25	202.00
	203.88	9.95	162.00	62.00	88.50	138.00	35.60	194.00	20.10	219.50	8.50	197.00
$(NH_4)_2SO_4$ + Inh	200.55	5.40	202.50	23.10	203.00	32.20	179.00	54.00	158.00	80.00	122.00	127.00
	200.65	5.23	202.50	23.40	200.50	34.35	182.00	55.50	157.00	78.50	133.00	116.00
Canisteo												
$(NH_4)_2SO_4$	200.30	4.55	18.05	172.50	0.00	204.00	0.00	217.00	0.00	221.00	0.00	247.50
	207.30	6.85	19.25	174.50	0.00	201.00	0.00	214.50	0.00	228.00	0.00	238.50
$(NH_4)_2SO_4$ + Inh	201.52	7.53	147.00	39.35	124.50	65.00	36.95	157.50	0.00	210.00	0.00	224.00
	202.41	7.45	150.50	40.35	133.00	63.50	50.50	144.50	0.00	219.00	0.00	227.50

APPENDIX E. Effect of heavy metals on the accumulation of NH_4^+-N and NO_3^--N in selected Iowa soils treated with Stay-N 2000

Soil	Incubation time											
	0 d		7 d		14 d		30 d		45 d		60 d	
	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-
	$\mu g\ g^{-1}$											
	Cu											
Clarion												
$(NH_4)_2SO_4$	250.50	5.95	146.50	59.60	42.95	126.00	0.00	186.70	0.00	175.50	0.00	181.25
	251.00	6.30	150.50	51.45	42.80	125.00	0.00	184.00	0.00	183.45	0.00	181.50
$(NH_4)_2SO_4$ + Inh	251.00	5.75	196.50	16.85	191.00	22.35	169.50	58.10	68.59	120.00	0.00	171.15
	250.00	6.50	196.00	15.05	190.50	20.50	172.50	50.20	72.00	129.55	0.00	171.50
Okoboji												
$(NH_4)_2SO_4$	175.65	8.56	56.30	123.55	0.00	175.20	0.00	173.42	0.00	200.95	0.00	197.80
	173.00	8.48	52.70	113.35	0.00	175.15	0.00	175.41	0.00	197.80	0.00	197.00
$(NH_4)_2SO_4$ + Inh	166.40	8.60	123.30	57.30	94.90	90.30	20.95	159.04	0.00	182.90	0.00	198.60
	166.70	8.42	122.35	57.25	89.80	85.75	26.70	162.15	0.00	180.22	0.00	200.60
Nicollet												
$(NH_4)_2SO_4$	264.10	6.75	147.00	88.33	36.20	165.94	0.00	185.20	0.00	187.59	0.00	185.73
	263.00	6.60	146.50	87.63	30.05	162.49	0.00	183.95	0.00	186.79	0.00	184.23
$(NH_4)_2SO_4$ + Inh	255.60	7.25	253.00	23.03	204.50	27.39	200.50	30.15	199.50	41.99	178.00	60.43
	262.00	6.15	256.50	22.03	209.50	26.89	202.50	32.20	198.00	43.49	179.50	61.58
Canisteo										17.89		18.23
$(NH_4)_2SO_4$	207.35	8.93	48.55	159.78	0.00	206.04	0.00	217.70	0.00	187.29	0.00	201.33
	206.65	9.23	52.10	171.58	0.00	201.39	0.00	227.40	0.00	189.14	0.00	194.73
$(NH_4)_2SO_4$ + Inh	202.95	9.19	173.35	58.43	131.70	85.44	112.75	115.55	31.32	166.69	0.00	191.43
	203.80	9.02	179.50	56.98	146.55	83.89	105.25	117.43	27.84	163.54	0.00	203.23

Zn

Clarion												
$(NH_4)_2SO_4$	219.40	6.94	176.30	50.95	79.35	123.01	0.00	179.05	0.00	181.04	0.00	182.80
	220.70	6.34	168.55	50.90	77.80	115.13	0.00	176.56	0.00	188.85	0.00	174.68
$(NH_4)_2SO_4$ + Inh	222.55	6.99	214.05	12.25	210.15	14.54	208.80	13.13	203.25	15.96	108.50	53.07
	222.85	6.39	214.40	11.11	209.05	13.29	207.30	14.76	201.20	18.90	108.28	51.76
Okoboji										17.89		18.23
$(NH_4)_2SO_4$	268.35	9.09	116.80	111.00	7.88	164.20	0.00	180.54	0.00	180.55	0.00	183.00
	268.60	8.85	112.50	110.75	9.72	168.31	0.00	178.85	0.00	177.70	0.00	181.25
$(NH_4)_2SO_4$ + Inh	270.35	9.68	221.05	36.90	155.05	53.49	125.62	68.00	98.15	95.61	59.80	149.20
	272.50	9.71	225.10	38.27	151.30	53.15	129.65	68.53	98.55	98.70	53.30	144.37
Nicollet												
$(NH_4)_2SO_4$	196.20	7.52	117.95	90.45	25.89	148.15	0.00	165.02	0.00	181.04	0.00	182.55
	193.30	7.42	118.35	89.41	21.13	148.79	0.00	163.14	0.00	178.81	0.00	171.87
$(NH_4)_2SO_4$ + Inh	195.15	7.51	227.25	14.20	233.20	12.45	234.10	14.53	225.75	15.96	204.20	44.48
	207.25	7.79	231.30	13.20	232.60	14.41	233.20	18.49	236.75	18.90	201.55	48.39
Canisteo												
$(NH_4)_2SO_4$	200.03	7.42	45.10	142.00	0.00	180.89	0.00	191.65	0.00	184.60	0.00	198.61
	200.24	7.95	46.65	143.90	0.00	179.08	0.00	197.20	0.00	192.97	0.00	197.15
$(NH_4)_2SO_4$ + Inh	201.35	7.67	164.15	55.18	142.85	68.12	98.05	108.48	56.19	126.75	9.32	184.07
	203.31	7.63	161.45	54.30	139.10	69.30	105.00	106.30	53.93	122.10	13.03	188.80

Pb

Clarion												
$(NH_4)_2SO_4$	209.02	6.75	112.00	10.30	33.60	139.50	0.51	175.00	0.00	195.50	0.00	204.50
	212.00	6.95	114.00	11.45	29.90	141.00	0.83	170.00	0.00	211.00	0.00	196.00
$(NH_4)_2SO_4$ + Inh	202.00	7.30	159.50	16.85	159.00	13.25	159.50	18.00	127.50	56.00	150.50	28.15
	203.31	7.30	161.00	15.05	158.50	13.35	156.50	19.05	130.00	56.50	153.00	30.60
Okoboji												
$(NH_4)_2SO_4$	228.31	9.55	35.30	135.50	0.00	172.00	0.00	195.50	0.00	187.00	0.00	177.50
	225.43	10.85	40.35	131.50	0.00	171.00	0.00	183.00	0.00	198.00	0.00	193.50
$(NH_4)_2SO_4$ + Inh	231.40	9.45	115.00	46.60	112.50	63.00	74.00	105.00	35.08	188.50	21.05	162.00
	228.21	9.45	127.00	47.05	106.50	66.50	69.50	107.00	34.78	204.00	30.25	157.50
Nicollet												
$(NH_4)_2SO_4$	199.23	5.25	110.00	18.51	43.08	149.41	0.00	189.11	0.00	186.47	0.00	212.39
	193.30	5.31	112.30	19.66	49.55	150.91	0.00	184.11	0.00	185.67	0.00	227.89
$(NH_4)_2SO_4$ + Inh	195.15	7.83	169.25	25.06	167.78	23.16	159.50	32.11	127.50	40.87	150.50	72.89
	197.24	7.56	161.00	23.26	165.71	23.26	156.50	33.16	130.00	42.37	153.00	73.39
Canisteo												16.89
$(NH_4)_2SO_4$	200.00	10.55	40.00	143.71	0.00	181.91	0.00	209.61	0.00	186.17	0.00	203.89
	201.00	11.21	40.35	139.71	0.00	180.91	0.00	197.11	0.00	188.02	0.00	214.89
$(NH_4)_2SO_4$ + Inh	201.35	9.32	125.00	54.81	112.50	72.91	64.00	119.11	23.21	165.57	11.35	205.39
	199.85	10.01	127.00	55.26	116.31	76.41	69.50	121.11	22.11	162.42	14.29	220.89

Cd

Clarion												
$(NH_4)_2SO_4$	219.40	6.00	152.00	36.15	123.50	71.00	39.05	155.50	10.65	184.00	0.00	206.50
	220.70	5.99	158.00	31.35	114.00	79.50	45.70	149.50	14.80	181.50	0.00	203.00
$(NH_4)_2SO_4$ + Inh	222.55	7.28	155.00	9.65	156.00	11.15	174.50	11.20	183.00	11.80	182.50	11.15
	222.85	7.30	174.00	9.85	167.50	11.45	183.50	11.10	155.50	12.95	173.50	13.15
Okoboji												
$(NH_4)_2SO_4$	268.35	10.45	93.00	99.50	24.95	162.50	0.00	205.00	0.00	189.00	0.00	210.00
	268.60	10.85	102.50	90.50	24.40	164.00	0.00	202.00	0.00	216.50	0.00	211.00
$(NH_4)_2SO_4$ + Inh	270.35	9.65	159.50	35.45	150.50	45.00	147.00	60.00	93.50	100.00	102.50	78.00
	272.50	9.45	161.00	37.95	155.50	51.50	139.00	68.00	104.00	87.50	113.00	92.00
Nicollet												
$(NH_4)_2SO_4$	196.20	6.25	181.00	26.77	169.50	30.62	114.00	87.81	55.50	140.27	28.95	150.34
	193.30	6.28	182.50	27.09	163.00	32.17	125.00	92.06	71.50	144.77	21.15	155.84
$(NH_4)_2SO_4$ + Inh	195.15	7.54	189.50	9.900	191.00	18.42	196.50	30.40	198.00	36.02	201.00	51.76
	207.25	7.56	189.50	9.720	192.50	18.27	198.00	30.72	198.00	44.97	205.00	51.15
Canisteo												
$(NH_4)_2SO_4$	200.03	11.35	147.50	21.37	88.00	69.87	0.00	137.81	0.00	143.77	0.00	152.34
	200.24	12.31	147.00	22.27	82.00	76.37	0.00	141.31	0.00	145.77	0.00	156.84
$(NH_4)_2SO_4$ + Inh	201.35	8.35	165.50	10.89	158.50	22.82	157.00	50.41	5.25	58.17	155.00	60.36
	203.31	10.45	175.00	10.93	166.00	27.37	160.00	48.11	4.42	63.12	155.50	64.34

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